SERUM AUTOANTOBODIES BLOCK CANCER INVASION IN 3D ORGANOTYPIC CULTURE

By:

Veena Padmanaban

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ABSTRACT:

Autoimmune diseases are caused at least in part by autoantibody-mediated immune responses. Several studies have reported altered risks for developing cancer in patients with autoimmune diseases. In breast cancer patients, the appearance of autoantibodies is temporally associated with the onset of malignant disease (Shah et al., 2010). However, the function of these autoantibodies in tumor progression remains unclear. Recent work from our laboratory shows that breast cancer invasion is led by specialized cancer cells that express cytokeratin-14 (K14). Interestingly, preliminary data from the Rosen laboratory detected autoantibodies against K14 in the serum of patients with autoimmune disease. Based on these data, we raised the specific hypothesis that autoantibodies directly limit tumor progression and metastatic spread by binding to tumor antigens on invasive cancer cells. We show here that purified IgGs from the sera of lupus, dermatomyositis and scleroderma patients bind human mammary tumor antigens and some of these autoantibodies (13200, 12106, and SLE1269) reduce tumor invasion in a 3D organotypic culture system. Furthermore, autoantibodies from patients with no cancer are more effective at blocking tumor invasion when compared to corresponding antibodies from cancer patients. The work described in this thesis presents the first experimental evidence implicating therapeutic roles of patient-derived autoantibodies in blocking breast tumor invasion. Additionally, targets of these antibodies may provide lead candidates for therapeutic targeting of breast cancer metastasis.

ACKNOWLEDGEMENTS

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CHAPTER 1: INTRODUCTION

1.1 Autoimmune Diseases

An autoimmune disease is a clinical syndrome in which an active immune response by T cells and/or B cells affects one's self-tissues. With the exception of rheumatoid arthritis, most of these diseases are rare and affect only 5% of the population of the western countries (Sinha et al., 1990).

1.1.a. Autoimmunity

Despite some progress, autoimmune diseases remain a challenging field to study and their precise causes remain poorly understood. For several years, immunology was founded on the principle that our immune cells (mainly T and B cells) recognize only foreign antigens and remain inactive towards self-tissues (Kyewski and Klein, 2006). In line with this, during lymphocyte development, genes encoding antigen receptors are randomly rearranged and the lymphocyte is exposed to antigenic signals from self-tissues. Immune cells that exhibit weak interactions with self-antigens are considered stimulatory and suitable for mounting immune responses against foreign pathogens, a process called positive selection (Kyewski and Klein, 2006). On the other hand, negative selection induces programmed cell death in immune cells that display high-affinity interactions with self-antigens (Kyewski and Klein, 2006). However, we now acknowledge that this process of negative selection is 'leaky' and some T cells with a high affinity for self-antigens can escape deletion and migrate to the periphery (Danke et al., 2004). At the periphery, there are several cell-intrinsic or cell-extrinsic regulatory mechanisms that

prevent activation of these escapees. Some of these mechanisms include induction of anergy, production of down-regulatory cytokines by regulatory T cells or interference with receptor signaling pathways (Seddon and Mason, 2000). Since this escape occurs physiologically in normal individuals, it is challenging to understand how this process becomes pathologic resulting in autoimmune disorders.

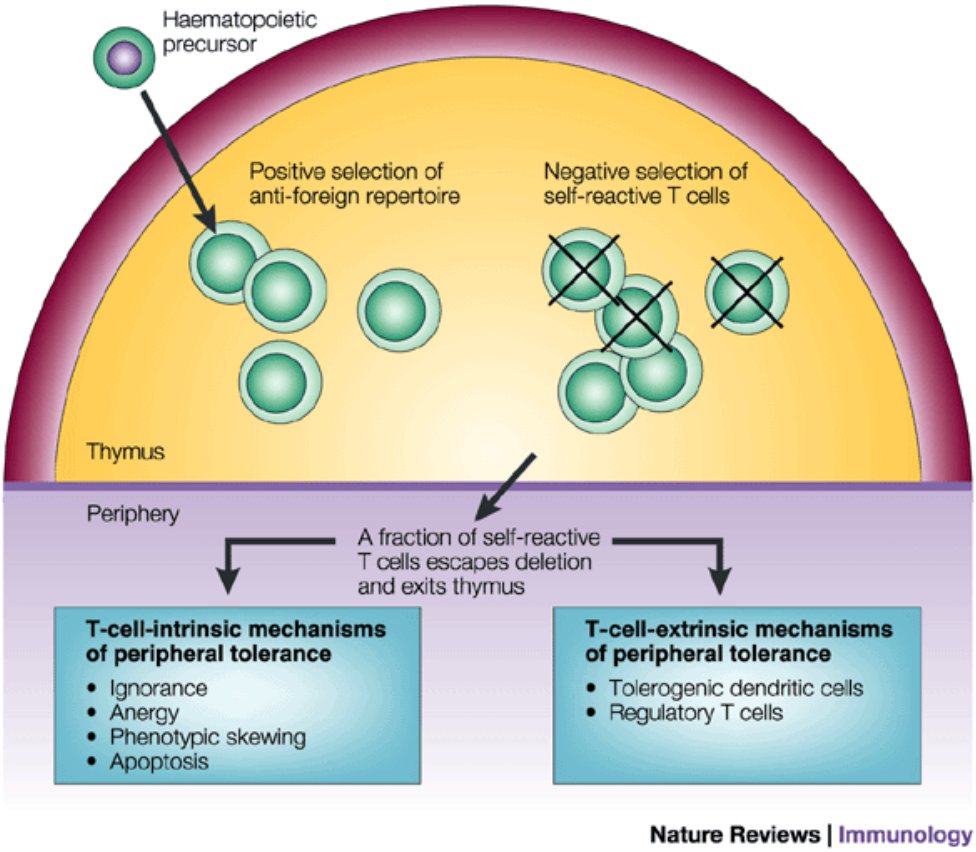


Fig 1: Schematic describing cell fate of a T cell that escape negative selection. Hematopoietic precursor cells undergo positive or negative selection based on their interactions with self-antigens. Fraction of self-reactive T cells escape negative selection and are controlled by peripheral tolerance mechanisms which could be T-cell intrinsic or T-cell extrinsic. (Walker and Abbas, 2002)

1.1.b Autoantibodies in Normal Physiology and Pathobiology

The immune system of healthy individuals also contains self-reactive T and B cells (Filion et al., 1996). Antibodies circulating in the serum of normal individuals, in the absence of any known immunization with the target antigen, are called natural autoantibodies. Natural antibodies consist of predominantly IgM, IgG and IgA immunoglobulins encoded by germline genes that exhibit a low-affinity for self-antigens (Tomer and Shoenfeld, 1988). Some of these antigens include nuclear proteins, DNA, cytoskeletal proteins, intracellular and membrane proteins (Coutinho et al., 1995). In contrast to the antigen-specific antibodies that are secreted by mature B cells in a T-cell dependent pathway in response to a foreign pathogen (Muramatsu et al., 1999), natural antibodies are not antigen specific towards pathogens and are produced by innate-like B cells in a T-cell independent manner (Avrameas, 1991). It has been estimated that 5-15% of splenic B cells secrete natural autoantibodies. These antibodies are believed to be conserved through evolution, suggesting that they are important in normal physiology for maintaining tissue homeostasis (Coutinho et al., 1995). Major functions of these autoantibodies involve scavenging for metabolic waste and senescent cells (Grabar, 1975), protection against viral and bacterial infections (Ochsenbein et al., 1999), and control of autoimmune diseases (Cohen and Cooke, 1986). Owing to its flexible antigen binding domain and pentameric structure, natural IgMs bind non-specifically to a wide range of microbes and activate the complement cascade (Ehrenstein and Notley, 2010).

There are several autoantigens including DNA and myelin proteins that are recognized by natural autoantibodies and are also targeted during the progression of autoimmune diseases like lupus (Cohen, 2000). Thus, it could be imagined that autoimmune disorders arise simply due to the deregulation of natural autoantibodies (Lacroix-Desmazes et al., 1998).

1.1.c Causes of Autoimmune Diseases

The etiology and pathogenesis of autoimmune diseases remains largely unknown. Genetic factors are considered to be a crucial determinant of susceptibility to autoimmune disorders (Invernizzi, 2007) where there seems to a familial clustering in patients. Furthermore, the rate of autoimmune disease concordance is at least four times or higher in monozygotic twins than in dizygotic twins (Cooper et al., 1999). Like most other diseases, autoimmune diseases are multigenic, that is, development of autoimmunity in patients occurs when multiple genes work together to produce an abnormal phenotype (Encinas and Kuchroo, 2000). Even in a genetically predisposed person, some form of external trigger — either in the form of an environmental exposure or a change in the internal environment — is usually required to stimulate an autoimmune response. The importance of these environmental triggers can be seen in studies of genetically similar populations in different environments. For instance, the incidence of type I diabetes and multiple sclerosis in a specific population is altered significantly as individuals migrate to different regions (Noseworthy et al., 2000).

1.1.d. Examples of Autoimmune Diseases

Dermatomyositis

Inflammatory myositis, which refers to the inflammation of muscle, is used to describe dermatomyositis, polymyositis, necrotizing myopathy, and inclusion-body myositis. Dermatomyositis affects 5 in every 100,000 persons in the United States (Furst et al., 2012). Common symptoms include a characteristic rash and muscle weakness which developed within a few weeks. The pathology of the disease includes binding of immune complexes to endothelial cells, activation of the complement system and subsequent lysis, leading to a decreased number of capillaries in the muscle (Mammen, 2010). Autoantibodies preferentially associated with dermatomyositis include those recognizing Mi-2, MDA5, TIF1γ, and NXP-2 – each associated with a distinct phenotype (Shah et al., 2015).

Scleroderma

Scleroderma is a systemic autoimmune disease best characterized by the hardening of the skin due to the increased synthesis of collagen leading to abnormal connective tissue (Fleming and Schwartz, 2008). The overall incidence rate of scleroderma in the adult population of the United States is approximately 20 per million per year (Mayes et al., 2003). Most patients affected by the disease present with Raynaud's phenomenon – reduced blood flow resulting in discoloration of fingers and toes. The most common scleroderma-specific autoantibodies include anticentromere, anti-topoisomerase, and anti-RNA polymerase (Shah et al., 2015).

Lupus

Systemic lupus erythematosus (SLE) is a chronic and systemic autoimmune disease that affects joints, skin, lungs, kidneys, blood cells or heart and follows a relapsing and remitting course. The disease is characterized by a multisystem inflammation with the generation of autoantibodies including antinuclear, anti-ds DNA, anti-ribonucleoprotein, anti-Ro, and anti-La (Ippolito et al., 2011). The incidence of lupus is about 5-7 per 100,000 persons (Somers et al., 2014). Symptoms for the disease may vary between individuals, but usually include chest pain, fatigue, hair loss, mouth sores, fever with no cause, and/or a butterfly rash.

Fig 2: Characteristic rashes of dermatomyositis, scleroderma, and lupus (butterfly rash). (Image sources from left to right - http://en.wikipedia.org/wiki/Dermatomyositis; http://byebyedoctor.com/scleroderma/; http://www.webmd.com/lupus/ss/slideshow-lupus-overview)

1.2 Breast cancer

Breast cancer refers collectively to neoplasms arising from the tissue of the breast. Breast cancers are often an adenocarcinoma, which is a cancer of the gland tissues (Allison, 2012). Types of breast cancer include:

- **Ductal carcinoma in-situ (DCIS):** Cancer begins in cells lining the ducts, but these cells do not invade through the walls of the breast.
- **Invasive ductal carcinoma (IDC):** Most common type of breast cancer that begins with cells lining the duct, which then invade into the surrounding breast tissue.
- **Invasive lobular carcinoma:** This cancer starts with the cells lining the lobules and can spread to nearby lymph nodes or other parts of the body.
- **Inflammatory breast cancer:** Cancer cells in this rare subtype block lymph vessels in the skin which causes symptoms similar to those that may occur during inflammation.

1.2.a Occurrence, Progression and Patient Outcome

Breast cancer is the most common cancer in women in the United States and the leading cause of death in these women (United States Cancer Statistics: 1999–2011 Incidence and Mortality Web-based Report). Although breast cancer can occur at any age, it is more likely after age 40, and the risk increases with age. The number of breast cancer cases diagnosed every year is the highest among all cancers and accounts for about 12% of all new cancer cases, and 25% of all the cancers in women (Jemal et al., 2010). One in every

eight (12%) women in the United States run the risk of being diagnosed with invasive breast cancer during her lifetime. It is estimated that 243,190 new cases of breast cancer will be diagnosed in 2015, and more than 40,000 of these women will succumb to their disease. In 2014, there were more than 2.8 million women with a history of breast cancer in the U.S (American Cancer Society, Surveillance Research, 2014).

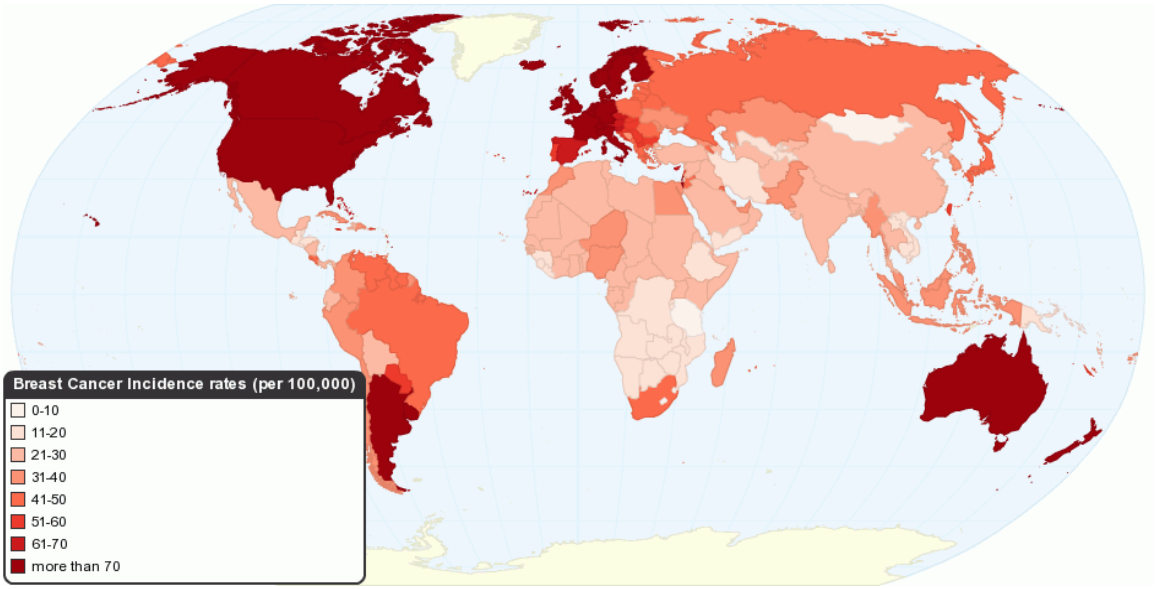


Fig 3: Heat map describing breast cancer incidence per 100,000 individuals, 2008.

(Source: http://chartsbin.com/view/yq6)

Breast cancer is a heterogeneous disease that develops in a continuum. The normal breast terminal end bud consists of lobules and ducts organized as a bi-layered epithelium of luminal and myoepithelial cells (Vargo-Gogola and Rosen, 2007). Atypical hyperplasia (ADH) is a pre-malignant lesion with abnormal cell layers in the duct or lobule. This progresses into DCIS which is characterized by abnormal cells. These cancer cells can then invade past the basement membrane in IDC, and once this happens the risk for

developing secondary tumors or metastases (MET) increases significantly (Vargo-Gogola and Rosen, 2007).

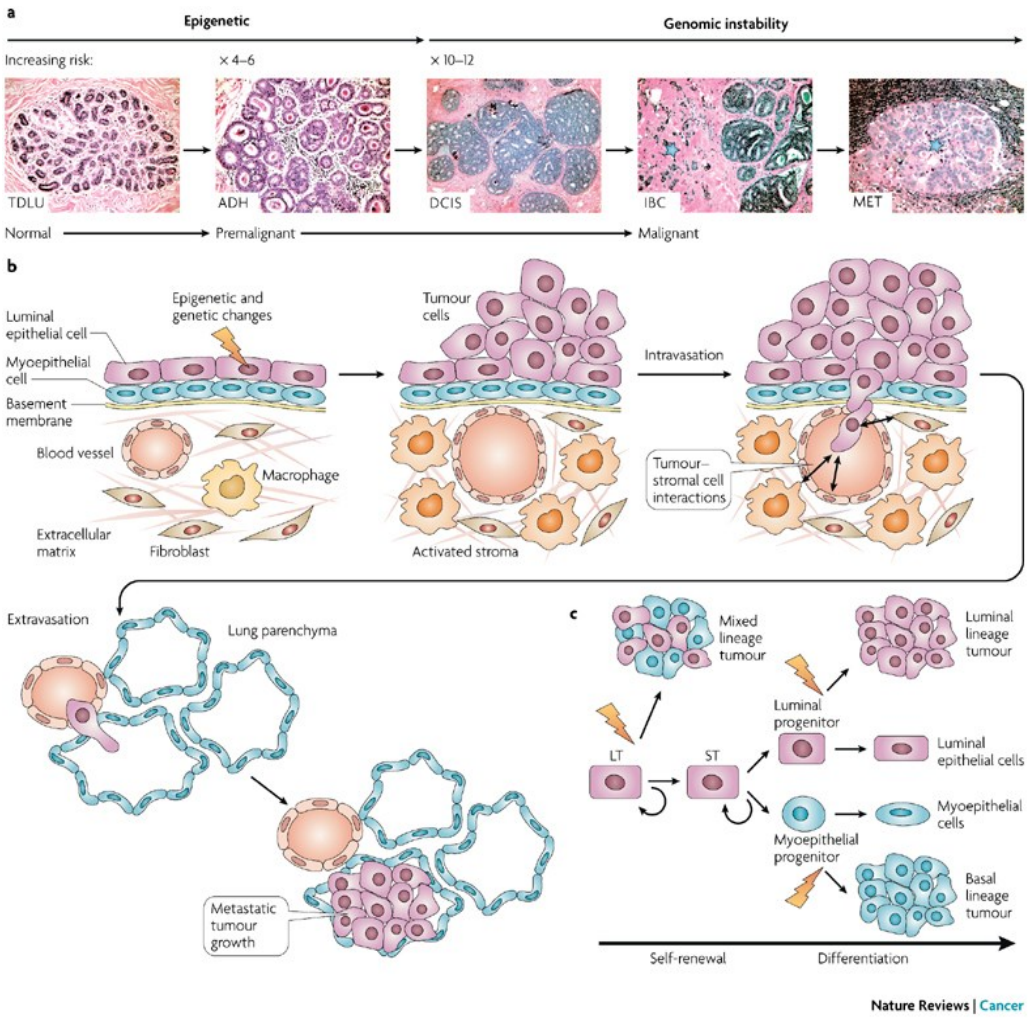


Fig 4: Breast cancer pathogenesis and progression. The normal breast epithelium consists of a bi-layered epithelium composed of luminal and myoepithelial cells arranges as lobules and ducts. Order of premalignant to malignant disease – ADH, DCIS, and IDC. Once cancer cells have escaped past the basement membrane in IDC, they can enter the vasculature and seed a secondary tumor at a foreign microenvironment. Progression to metastatic disease could occur due to combinations of genetic, epigenetic and environmental alterations (Vargo-Gogola and Rosen, 2007).

1.2.b Breast Cancer Treatment: Cancer immunotherapy

The overall 5-year survival rate for breast cancer has seen a dramatic improvement from 63% in the 1960s to 90% now (Berry et al., 2005). Patients with local, non-invasive disease have a very promising survival rate of 98%. Treatment options for breast cancer patients include surgery (lumpectomy – resection of the tumor and surrounding breast tissue, mastectomy – removal of entire breast, axillary lymph node dissection – removal of lymph nodes draining the breast), radiation therapy, hormone therapy, or chemotherapy (1998).

The modern treatment of breast cancer has evolved to include targeted approaches, with treatment based on the presence or absence of receptors for estrogen (ER), progesterone (PR), and HER2 (Tinoco et al., 2013). Almost 75% of breast cancers are ER-positive and majority of these malignancies respond to treatments that reduce circulating estrogens. Tamoxifen, an antiestrogen, completely binds the ER and inhibits estrogen binding (Miller, 2004).

An important principle in cancer immunotherapy is that the emergence of cancer represents a failure of the body to mount an effective immune response against cancer (Parish, 2003). Passive cancer immunotherapy uses antibodies to target specific antigens in the cancer: for example, the breast cancer drug Herceptin is an antibody that targets the HER2 receptor. Binding of the drug to the HER2 receptor on the surface of breast tumor cells prevents cell growth (Mathe, 1987). However, drugs against cancer proteins within cells have not been developed because antibodies are considered too large to pass through

the membrane of most cell types. Yet, evidence for antibodies having the potential to enter viable cells has been accumulating since 1978 (Alarcon-Segovia et al., 1978).

1.2.c Collective Cell Invasion and Metastasis

The spread of cancers, from their organs of origin to other sites within the body, a process called metastasis, constitutes a defining hallmark of malignancy (Hanahan and Weinberg, 2011). It continues to be the most difficult clinical problem complicating the treatment of cancer patients. About 20-50% of patients diagnosed with breast cancer eventually progress to metastatic disease (Lu et al., 2009). Despite advances in the treatment of early stage breast cancer, mortality from metastatic breast cancer has remained unchanged over the last 30 years (Tevaarwerk et al., 2013). The 5-year survival rate for localized disease is 98%, but the number falls to 22% for patients with distant metastasis (Cancer Research, UK). Thus, there remains a critical need for both an understanding of the mechanisms underlying metastasis and the development of therapeutic approaches to target the metastatic process.

Metastasis is a multi-step process that begins with cancer cell motility, invasion, dissemination, intravasation into a blood or lymphatic vessel, extravasation and colonization at a distant site (Chaffer and Weinberg, 2011). Only a very small set of tumor cells succeed at each of these steps, making metastasis a very inefficient process (Sahai, 2007). The acquisition of an invasive phenotype by tumor cells is one of the first steps of the process. Traditionally, invasion is defined as the penetration of healthy tissue by either single cells, groups of cells or strands of connected tumor cells (Leighton et al., 1960). For

breast cancer, the tumor border is commonly surrounded by a collective and invasive group of cells that express cell adhesion molecules like cadherins (Friedl et al., 2012). The morphology and organization of collectively invading cancer cells can vary considerably - ranging from strands of only one or two cells in diameter, to broad masses that can include cells that do not contact the extra-cellular matrix (ECM). It is believed that this collective cell behavior is likely to contribute to several steps of the metastatic cascade (Friedl et al., 2012).

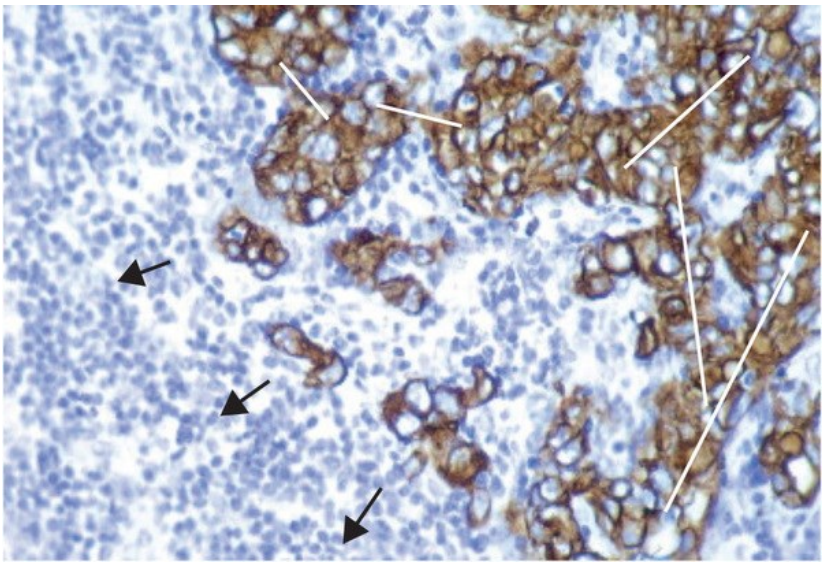


Fig 5: Collective invasion in a clinical tumor specimen. Cells within the tumor mass are interconnected (white lines), and smaller collective groups of cells have invaded into the surrounding stroma (direction of invasion marked by black arrows). (Friedl et al., 2012)

1.2.d Modelling breast tumor behavior in 3D organotypic culture systems

The inaccessibility of the relevant anatomical sites has hindered our ability to study metastasis. Despite that, we have made significant progress by developing three-

dimensional (3D) culture systems and advanced molecular tools that allow us to study mammalian biology in real-time.

Two-dimensional culture enabled biologists to observe and manipulate mammalian cells and laid the foundation for cell and molecular biology. However, these culture systems fail to emulate the three-dimensional (3D) architecture and organization of the tissue in relation to its surrounding microenvironment. Consequently, there is an incomplete understanding of mammalian biology at the tissue level (Shamir and Ewald, 2014). To overcome this barrier, we developed an ex-vivo organotypic culture system that allows us to study the epithelial biology of the mammary gland in an environment that closely resembles that *in vivo* (Simian et al., 2001). Organoids containing several hundred cells isolated from primary tissue are capable of reorganizing into their normal spatial configuration as observed *in vivo* when embedded in 3D ECM gels (Matrigel or Collagen). The choice of the microenviroment can dictate cell behavior of organoids (Nguyen-Ngoc et al., 2012). When normal mammary organoids are embedded in a basement membrane rich ECM (Matrigel), organoids undergo branching morphogenesis. While in collagen I, the same organoids exhibit robust transient migration and dissemination (Nguyen-Ngoc et al., 2012).

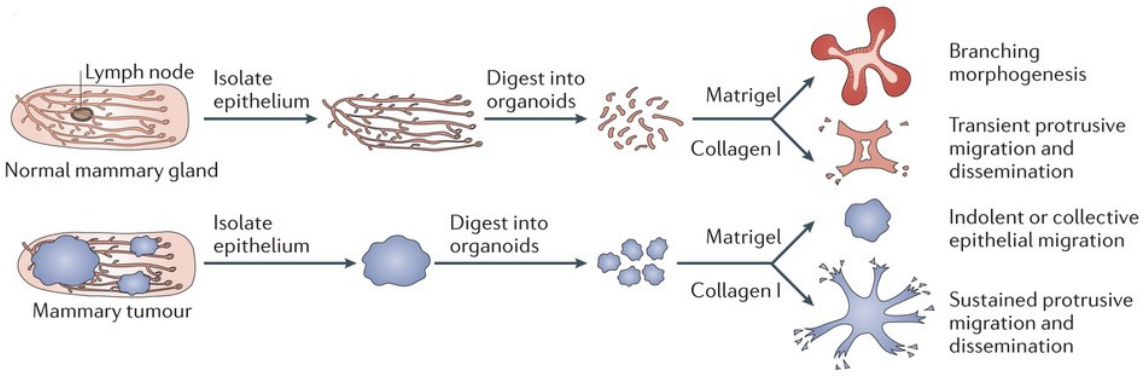


Fig 6: The microenvironment regulates epithelial behavior. Organoids generated from the same mammary tissue (normal gland or tumor) show altered invasive and disseminative behaviors when exposed to different microenvironments – Matrigel or Collagen I. (Shamir and Ewald, 2014)

Collective cancer cell invasion can be observed in similar 3D matrix-based organotypic cell cultures. In such ex-vivo cultures, collective invasion observed in vivo could be correlated to migration of multicellular groups of cells within organoids while retaining their cadherin based cell junctions (Ewald et al., 2008). In the Ewald lab, we mechanically and enzymatically digested mammary tumors from mice to generate clusters of 100-300 tumor cells called organoids. To study collective invasion, we embed these organoids into a collagen I gel that closely resembles the tumor microenvironment of an invasive breast carcinoma. After 48-72 hours, organoids progressively extend multicellular strands into the collagen I. Our research group has previously identified specialized cancer cells that commonly lead collective invasion in breast cancers (Cheung et al., 2013). The cells leading these invasive stands or leader cells express basal epithelial genes including keratin-14 (K14). Luminal cancer cells convert phenotypically into these leader cells upon induction

of K14 expression. Importantly, a knockdown of K14 was sufficient to block collective invasion in culture and in vivo (Cheung et al., 2013).

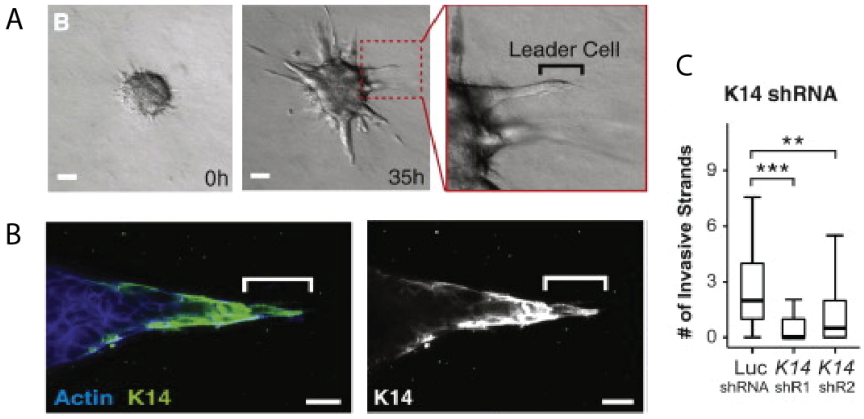


Fig 7: Collective invasion is led by K14+ cells. Organoids generated from mammary tumors of a mouse model of breast cancer collectively invade into collagen I gels. These invasive strands are led by K14+ cells. K14 expression is required for efficient metastasis formation. (Adapted from (Cheung et al., 2013))

1.3 Autoimmunity and Cancer

1.3.a. Cancer Immunosurveillance and Immunoediting

It was proposed as early as the 1950s that mutations in cancer might provoke the immune system to mount a response, causing tumor regression (Burnet, 1957). However, this hypothesis faded after numerous experiments in immunocompromised mice failed to show an increase in cancer development when exposed to a mutagen (Stutman, 1975). However, in the last few decades we now recognize the importance of the immune system is controlling cancer. Natural killer cells and T-cells are essential components of this process and recognize 'stress' and 'danger' signals presented by tumor cells (Diefenbach and Raulet, 2002). Instead, we now recognize that several components of the immune system such as IFN gamma and perforin, are required for regulating cancer initiation (Street et al., 2001). The immune system is effective in recognizing certain tumor antigens, making the tumor immunologically active.

Tumors are constantly modulated by the immune system such that the resulting tumor is less capable of stimulating a host immune response, a process called immunoediting (Dunn et al., 2002). Common forms of immunoediting include loss of expression of tumor antigens by the tumor cells to avoid immune detection, or expression of immune checkpoints by the cancer (Pardoll, 2012).

Based on the relative dominance of the cancer or the immune response, cancer immunoediting has three stages – elimination, equilibrium, and escape (Schreiber et al., 2011). During the elimination stage, the immune system dominates and cancer is

eliminated. In the equilibrium stage, the cancer and the immune responses are balanced such that neither does the cancer advance significantly, nor does the immune system beat the cancer. The third stage of escape represents the unregulated cancer growth (Screiber, 2011).

1.3.b Association of Autoimmune Diseases and Cancer

Connections between autoimmune rheumatic diseases and cancer have been becoming increasingly evident over the last few decades. Although a clear cause for autoimmune diseases has not been identified, it is believed that the dynamic interactions between cancer and the immune system may be relevant to understanding the origins of autoimmunity (Schreiber et al., 2011). These interactions are bidirectional; the success of immune checkpoint inhibitors as potent anti-cancer therapies, suggest that natural immune responses to cancer occur and could regulate the emergence of cancer.

In some cases, autoimmune antibodies may be produced as a result of an autoantigen mutation in the patient's cancer. In a subset of scleroderma patients with RNA Polymerase III (RNAPol III) autoantibodies, malignancy was detected in a close temporal association with the clinical onset of autoimmune disease (Joseph et al., 2014). Tumors from these patients had genetic alterations in the gene encoding RNAPol III. In contrast, patients with autoantibodies against other antigens did not have mutations in the RNA Pol III gene. This data suggests that expression of RNAPol III autoantibodies in cancers might be specific to scleroderma immune responses (Joseph et al., 2014). Although this immune response against RNAPol III was initiated against the mutated protein, the immune

response spreads to the wild type version, causing injury to self-tissues. Perhaps, in RNA polymerase III-positive scleroderma patients with no detectable cancer (~80% of scleroderma patients), the immune system successfully eradicated any cancer that may have arisen (Joseph et al., 2014). This idea strongly implicates the importance of the immune system in maintaining cancers in the elimination stage and forms the basis for the hypothesis of this thesis work.

Similar studies in patients with dermatomyositis suggest that patients with a malignancy have cancer up to 2 years prior to myositis diagnosis (Hill et al., 2001). Patients with myositis-specific autoantibodies have a significantly lower risk of an associated cancer compared to autoantibody negative myositis patients (Chinoy et al., 2007). Recent reports indicate that cancer therapy may improve outcomes in myositis, implicating a mechanistic relationship between cancer and the autoimmune disease. Unfortunately, investigating the temporal association between cancer and autoimmune diseases is complicated because of the variations in clinical phenotypes, tumor types, age of onset of autoimmune disease, cancer and rheumatic therapies used on the patient.

1.3.c. Model for cancer-induced autoimmunity

Transformation of normal cells could occasionally result in the mutation of existing autoantigens. These mutations are secondary mutations in a subset of cells, and thus, not all cancer cells will have them. The first immune response would be mounted against this mutated form of the antigen, and may eventually spread to the wild-type version due to epitope spreading. During this process, auto-reactive T cells become activated in response

to self-epitopes released due to tumor-specific T cell-mediated damage. Immune cells are now capable of destroying cancer cells with the mutation, and also cancer and other normal self-tissues that express the wild type antigen. Once initiated, this autoimmune response is self-propagating, and results in significant tissue damage (Shah et al., 2015). In patients with autoimmune diseases that do not present with a malignancy, the immune system could be hypothesized to have successfully eradicated the cancer.

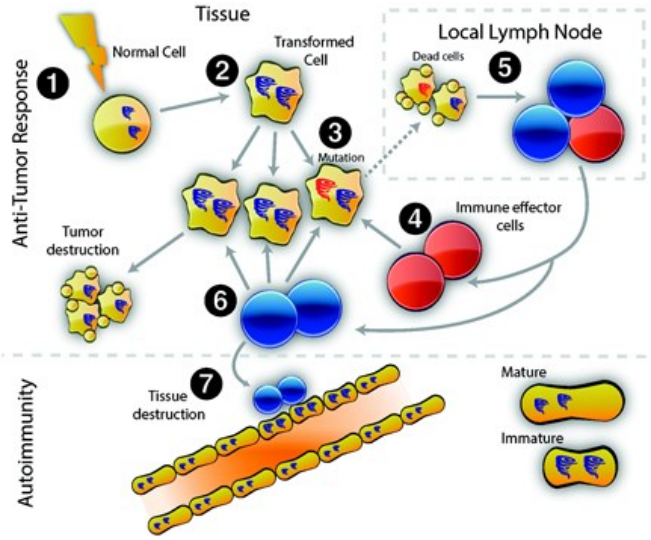


Fig 8: A model for cancer-induced autoimmunity. Transformation of normal cells could mutate autoantigens. Immune response initially targets cancer cells with these mutations but eventually spreads to those with the wild-type version as well. Effector cells targeting with wild-type alleles also cross-react with healthy tissues, resulting in autoimmune disease. (Shah et al., 2015)

1.3.d. Autoantibodies and Cancer

Autoantibodies are commonly detected in the serum of cancer patients. For example, antibodies against p53 and p16 were detected in 10–20 % of patients with

various types of cancers (Soussi, 2000). The identification of circulating biomarkers of early stage malignancy is a critical component of ongoing efforts aimed at reducing the overall burden of human cancer through early detection.

Antibodies are a critical part of our immune response against foreign antigens. Antibody dependent cellular cytotoxicity is a mechanism by which effector cells of the immune system lyse a target cell with antibodies bound to its membrane antigens. Antibodies bound on the cell surface of a pathogen also activates the complement system and causes subsequent pathogen destruction (Seidel et al., 2013). It was reported in 1978 that an autoantibody to nuclear ribonucleoprotein from the serum of a lupus patient penetrates the nuclei of live human mononuclear cells through Fc receptors (Alarcon-Segovia et al., 1978). Such penetrated autoantibodies can have varied effects on the cell expressing the antigen ranging from no effect, induction of apoptosis, or mitotic arrest (Alarcon-Segovia, 2001). It was recently shown that a cell-penetrating lupus antibody, 3E10, inhibits DNA repair, and as a single agent is lethal to BRCA-2 deficient human cancer cells (Hansen et al., 2012). Although patients with lupus are at an increased risk for developing cancer, they have a lower incidence of breast and ovarian cancers compared to control populations (Bernatsky et al., 2011). Based on this, it could be hypothesized that circulating autoantibodies in lupus patients provides protection against the development of BRCA-mutated tumors.

1.4 Hypothesis and Rationale for Thesis:

The cause of autoimmune diseases is still widely debated in the scientific community. Recent evidence suggests that a malignancy can drive the emergence of certain autoimmune diseases in patients. Cancer and autoimmune disease is diagnosed around the same time point in scleroderma patients with POLRA3 autoantibodies in their serum (Joseph et al., 2014). It is believed that the immune system is very active during the initial stages of cancer, and can succeed in keeping the cancer at bay. This is expected to be the case in patients with scleroderma and RNA Pol III autoantibodies where a malignancy doesn't develop.

The laboratory of Dr. Antony Rosen has identified autoantibodies against K14, a protein we have shown to be important for collective invasion in breast cancer, in patients with dermatomyositis. There are several recent reports that suggest malignancy is identified in patients with dermatomyositis prior to the clinical onset of the autoimmune disease (Hill et al., 2001). Thus, autoantibodies generated in these patients could represent the involvement of the immune system in fighting the cancer by developing antibodies against an antigen critical to tumor progression (K14).

Based on these preliminary studies, we initiated a collaboration with Dr. Antony Rosen to study the function of autoimmune antibodies in regulating collective cancer invasion. The aim of this thesis project is to evaluate the role patient derived autoantibodies in regulating collective tumor invasion in 3D organotypic culture systems.

CHAPTER 2: MATERIALS AND METHODS

Purification of Autoantibodies from Human Serum

Blood was collected from healthy controls or patients with autoimmune diseases (dermatomyositis, lupus, or scleroderma). To avoid the dilution of serum samples, zebra spin desalting columns (89882; Life Technologies) were used to perform buffer exchange into sodium phosphate for the subsequent steps. For purifying the total IgG from the serum, the flow through was collected from running the sample through Melon gel IgG spin purification kits (45206; Life technologies). The IgG concentrations of the sample was then estimated by coomassie staining of samples that were electrophoresed on SDS-PAGE gels. For estimating the standard curve, rabbit IgG samples of 5, 10, 20, 40 and 80ug were run on each of these gels. Purified IgGs were then stored at -80^0C.

Isolating Human Tumor Organoids

Using mechanical disruption, enzyme digestion, and centrifugation we purified fragments of primary mammary tumors called tumor organoids. Human tumors arrive from the Cooperative Human Tissue Network (CHTN) in DMEM. The media is washed out, tumors are then treated very briefly with 5mL of fungizone solution: 10ml Pen-Strep (P4333; Sigma), 10ml Fungizone (15290-018; Life Technologies), and 500 ml DPBS (D8662; Sigma). Primary breast tumors from patients with metastatic disease were minced with a scalpel and digested for 1 hour at 37°C in collagenase solution: (DMEM (10565–018; Gibco) with 2 mg/ml collagenase (C2139; Sigma-Aldrich), 5% FBS (F0926; Sigma-Aldrich), 5 μg/ml insulin (I9278; Sigma-Aldrich), GlutaMAX (35050-079; Gibco) and Penicilin-

Streptamycin (15140-122; Gibco)). The suspension was centrifuged at 1500 rpm to remove adipocytes, and the pellet was treated with 2U/u L DNase (D4263; Sigma-Aldrich) to separate out organoids. Single cells were removed using four quick spins at 1500 rpm, and the solution was enriched for organoids. Organoids were embedded in collagen I gels at a density of 1-2 organoids/μl and plated as 100-μl suspensions in 24-well (662892; Greiner Bio-One) or 8-well (154534; Lab Tek) coverslip-bottomed plates over a 37°C heating block. Gels were allowed to polymerize for 30 min at 37°C and then cultured in human tumor mammary epithelial medium: 100ml DMEM with 4500 mg/L glucose, sodium pyruvate, and sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture (D6546 Sigma; 1 mL of 100X GlutaMAX (35050-061 Life Technologies); 100 U/ml / 100ug/ml Pen-Strep (1ml of Pen 10,000 U/ml/ Strep 10,000 ug/ml) (P4333 Sigma); 10 mM Hepes Buffer, pH 7.3 (1ml of 1M stock) (118-089-721 Quality Biological, INC.); 0.075% BSA (250ul of 30% stock) (A9576 Sigma); 10 ng/ml Cholera Toxin (1ul of 1mg/ml stock) (C8052 Sigma); 0.47 ug/ml Hydrocortisone (1ml of 50ug/ml stock in PBS) (H0396 Sigma); 5 ug/ml Insulin solution, human (125ul of 4mg/ml stock or 50 uL of 10mg/mL stock) (I9278 Sigma); 5 ng/ml EGF (5ul of 100ug/ml stock) (E9644 Sigma) for six days.

Preparation of Collagen Gels for Culturing Tumor Organoids

Tumor organoids isolated from primary human breast tumors were embedded in collagen solution prepared from rat tail collagen I (354236; Corning*)* using the following recipe. Combine 375uL of 10X DMEM (D2429; Sigma) and 100uL of NaOH (S2770; Sigma*)* and mix well until the solution turns to a dark pink color. Add 3.5mL of collagen I and mix well until the color remains stable. *As the pH changes from acidic to neutral to basic, the*

solution changes color from yellow to light pink/ orange to dark pink. The solution was titrated with small volumes of NaOH until the desired color of light pink or salmon was attained. All steps were performed on ice. Allow this solution to polymerize on ice for 1-2 hours until the solution turns cloudy.

Culturing Human Tumor Organoids with Serum Autoantibodies

Tumor organoids collected on day 0 (day of plating them in culture) were fixed in 4% paraformaldehyde solution for 15 minutes. The remaining organoids were allowed to grow in culture medium with or without 10% of a specific autoantibody* (225uL of culture medium + 25uL of autoantibody) for 6 days, or until the organoids were invasive into the surrounding collagen I gels. These gels were then fixed in 4% paraformaldehyde solution for 15 minutes and analyzed for their levels of invasion. (* - variations of this experiment were also performed at 4% culture volume, and at a constant autoantibody concentration of 150ug/mL or 1uM.)

Immunofluorescence

Tumors organoids in collagen I gels were harvested at day 6 of culture and fixed for 15 minutes in 4% paraformaldehyde. They were then embedded in Tissue Tek® Optimal Cutting Temperature compound (O.C.T., Sakura) and frozen at -80°C overnight. O.C.T blocks were sectioned at 50 micron thickness using a Leica cryostat (Leica Biosystems, Germany) set to -27°C. For antibody staining, the O.C.T was removed by rinsing with PBS for 45 minutes. Samples were blocked for 2 hours with 10%FBS/1%BSA/PBS solution, incubated with the serum autoantibodies (50ug/ml) diluted in a 1%FBS/1%BSA/PBS

solution overnight at 4°C. Samples were rinsed with PBS for 30 minutes. Slides were incubated with goat anti-human secondary antibody (A-11013; Life Technologies) diluted at a 1:200 ratio in a 1%FBS/1%BSA/PBS solution for three hours. Samples were rinsed with PBS for 30 minutes, mounted with Fluoromount (F4680; Sigma), and sealed with coverslips.

DIC Microscopy

Differential interference contrast (DIC) imaging of tumor organoids was conducted using an an LD Plan-Neofluar 20×/0.4 Korr Ph2 objective lens and a Cell Observer system with a Zeiss AxioObserver Z1 and a AxioCam MRM camera (Carl Zeiss, Germany). Photoshop CS6 and ImageJ were used as needed to adjust levels and gamma for each channel on entire images to maximize image clarity.

Confocal Microscopy

Confocal imaging of fixed tumor sections was conducted with a Zeiss 780 laser scanning confocal microscope (Carl Zeiss, Germany). A 40× LD LCI C-Apochromat objective lens (Carl Zeiss) was used for high magnification image acquisition with water used as the imaging medium. Acquisition of fixed images was performed using Zen 2011.

Scoring Criteria for Invasion and K14 Intensity

Invasion Scoring: Score b: The organoid cannot be evaluated or not an organoid. Score d: The organoid contains only cells that are rounded up. If there are small clusters, the organoid is not dead. Score 0: Organoid has rounded borders. Score 1: Organoid has at

least one protrusive cell or a wide front with no clear leaders or has an invasive strand but the tip is out of focus. Score 2: Organoid has two or fewer invasive strands (defined as containing at least 4 cells in the strand, even if the tip of the strand is blunt). Score 3: Organoid has three or more large invasive strands.

K14 intensity scoring: Score 0: Organoid is negative for K14. Score 1: Organoid has a weak diffuse staining or less than 5% focal K14 positive cells. Score 2: Organoid has bright diffused staining or greater than 50% of its surface that is K14 positive. Score 3: Organoid has greater than 75% surface with K14 positive cells where individual cells are clearly identified.

CHAPTER 3: RESULTS

3.1 An Ex-Vivo 3D Culture System for Monitoring Invasion of Human Tumor Organoids

Breast cancers use several mechanisms to invade into surrounding tissues, yet our understanding of the spectrum of invasive behaviors in human breast tumors is limited. Based on a 3D organoid assay previously developed (Cheung et al., 2013), we identified K14 positive leader cells participating in the process of collective invasion in tumors from mice models of metastatic breast cancer. We have now successfully developed and optimized an efficient protocol for isolating tumor organoids from typically 0.2g of tumor sample obtained at the time of patient surgery (Fig 1A, B).

We processed 70 human breast tumors (received from the Cooperative Human Tissue Network), and retrieved organoids from 57 of these tumor samples (81% success rate). The protocol is now optimized to isolate a median of 1,333 organoids per gram of tissue received. These tumor organoids were embedded in collagen I gels to allow us to quantify their invasive behavior and to define the molecular phenotype of leader cells. When embedded in a collagen matrix, tumor organoids invade and extend several multicellular invasive strands (Fig 1C) (Nguyen-Ngoc et al., 2012). The leader cells of such invasive strands are positive for K14, a basal epithelial marker (Cheung et al., 2013).

In this cohort of 70 tumors, only ~30% of tumors invaded efficiently into fibrillar collagen, while the others failed to invade despite robust growth in culture (Fig 1D). Consistent with data from murine tumors, the cells leading the invasion front were K14+ for more

than 95% of leaders identified (Fig 1E). We scored the level of invasion for 1,981 organoids from 58 patient samples on a scale of 0, 1, 2, or 3 (Fig C). Similarly, we also scored 1,505 organoids isolated from 56 patient samples for K14 expression intensity on a scale of 0, 1, 2 or 3. We observed that the invasion efficiency of an organoid was strongly correlated with both the level of K14 expression (Fig 1F; n=911 organoids, 53 tumors, p value < 2.2x10-16) and induction of K14 expression in tumor cells (Fig 1G; n=45 patient samples, R-correlation = 0.795, p value = 6.52x10-11).

Apart from these tumor samples, we also received 11 normal mammary specimens from breast reduction surgeries. The tissue was processed in a manner similar to the tumors, and we were successful in isolating organoids from 73% of these samples. The mean isolation rate for normal mammary was 216 organoids per gram of tissue.

To summarize, we have successfully optimized a protocol for efficient isolation of tumor organoids from human breast tumors. We also reveal the spectrum of invasive behavior in patient-derived breast tumor samples, and validate that the K14 program is a common molecular program for collagen-dependent tumor invasion.

3.2 Serum Autoantibodies Show Specificity for Mammary Tumor Antigens

We obtained purified IgGs from the sera of patients with lupus erythematosus, myositis, and scleroderma. Organoids generated from human breast tumors were fixed on day 6 of culture, and stained with the eighteen autoantibodies provided by the Rosen Lab. As a control, we also stained tumor tissue with antisera collected from two normal patients (Fig 2A).

We observed positive staining in ten of the eighteen autoantibodies (56%) we tested against tumor tissue (Fig 2B, C). Of these, 100% (10/10) of the autoantibodies stained the cytoplasm of tumor cells and 50% (5/10) exhibited positive nuclear staining (Fig 2C). Among the different diseases, 75%, 60% and 44% of the dermatomyositis, lupus and scleroderma antisera stained tumor organoids (Fig 2B). In order to test if the autoantibodies specifically bind only to tumor antigens, we also compared these results with those obtained by staining organoids generated from normal mammary reduction tissue. In this case, we observed staining with seventeen of these autoantibodies (95%). It was surprising to detect binding of patient derived autoantibodies on normal mammary. 94.1% (16/17) of antisera which showed positive staining stained the cytoplasm and 47.1% (8/17) stained the nucleus (Fig 2C). Interestingly, while control antisera failed to stain the tumor tissue, they stained the normal mammary tissue.

We also scored the presence and intensity of staining as – or +, and + or ++ respectively (Fig 2C). We observed either cytoplasmic, nuclear or both cytoplasmic and nuclear staining with each antiserum (Fig 3A, B). While six antisera stain the normal and tumor

tissues with equal intensities, ten stain normal tissue better. Two other antisera (13200 and 12106) show a stronger binding to tumor organoids relative to those obtained from normal tissues (Fig 3A, B). This represents an interesting circumstance wherein the antibody could be binding to antigens expressed specifically by tumor cells.

Given the increased binding of autoantibodies to normal tissue, we asked if we were detecting immunoglobulin subtypes other than IgG. To test this, we used a secondary antibody specific to the Fc receptor of IgG and tested the ability of five control autoantibodies (isolated from healthy individuals) to bind organoids derived from a single normal mammary reduction tissue. Interestingly, binding patterns of autoantibodies did not alter significantly between IgG specific and non-specific secondary antibodies, suggesting that the detected signal was from IgGs (Fig 4).

Six autoantibodies (12106, 13040, 13142, 13200, SLE1269, and FW1366) among the panel of eighteen patient-derived autoantibodies showed significant binding against tumor antigens. We tested the reproducibility of the staining pattern of these autoantibodies against organoids generated from two other human mammary tumors. Tumor #1 was an ER+/PR+/HER2- with an invasive ductal histology, tumor #2 was a triple negative lobular carcinoma, and tumor #3 was an ER+/PR+/HER2- tumor with both invasive ductal and lobular characteristics. The staining pattern of the six autoantibodies varied significantly between each of these tumors. The differences were both in terms of signal intensity and localization (nuclear vs cytoplasmic or both) (Fig 5).

To further assess the ability of autoantibodies to regulate tumor growth, we obtained purified IgG from patients with autoimmune disease and a known cancer status. These IgGs were also paired for identical known autoantibodies to allow for easier comparison. 10015 and 9070 contained anti-TIF1 gamma antibodies, the former had no cancer, while the latter developed cancer. Similarly, 9109 and 7107 were anti-NXP2 containing sera from individuals who had no cancer and cancer respectively. We tested the ability of these autoantibodies to bind organoids generated from three human breast tumors – two ER+/PR+/HER2- tumors and one triple negative (ER-/PR-/HER2-) tumor. Overall, there seems to be no obvious trend differentiating staining patterns between cancer and no cancer autoantibodies. One anti-TIF1g autoantibody from a patient with cancer (9070), however, seemed to stain the nucleus, while the same autoantibody from a no cancer patient (10015) did not show nuclear staining (2/3 tumors) (Fig 6).

In conclusion, we present the first evidence that autoantibodies from patients with autoimmune diseases specifically recognize antigens expressed in human breast tumors.

3.3 Serum Autoantibodies Regulate Tumor Invasion in 3D Organotypic Cultures

We next asked if autoantibodies derived from patient sera could affect tumor progression in a 3D organotypic culture system. To address this question, we cultured organoids derived from human tumors along with autoantibodies. We selected four autoantibodies that gave interesting staining patterns with previous experiments – SLE1269, FW1366, 13200 and 12106. Each autoantibody was added at a concentration of 10% of the total culture volume.

We began by estimating the cytotoxicity of these antibodies to tumor organoids. A dying organoid loses its smooth borders and individual cells start rounding up. Based on this change in morphology, we assessed the cytotoxicity of each autoantibody. The control antibodies showed no cytotoxicity, while patient-derived antibodies caused minimal (4-17%) cell death (Fig 7B, C).

We next evaluated the effect of serum autoantibodies on tumor invasion. The tumor organoids generated from two primary breast tumors (both ER+/PR+/HER2-) were not invasive on day 0 (or the day of plating organoids in culture), but became invasive at day 6 of culture (Fig 7A). The baseline level of invasion of the two tumors in the absence of autoantibodies was 33.3% (9/27 of the organoids analyzed) and 8.3% (2/24 of the organoids analyzed) respectively (Fig 7C). Interestingly, in the presence of antibodies isolated from no disease controls (C3 and C34), invasion of organoids increased to 56.3% and 41.2% respectively for tumor #1 and 30% and 25% respectively for tumor #2 (Fig 7A, C). The patient-derived autoantibodies FW1366 increased tumor invasion to 57.1% for tumor #1

and 31.6% for tumor #2 (Fig 7A, C, D). Autoantibodies 13200 and 12106, on the other hand, decreased invasion in both tumors to 12.5% and 26.1% respectively for tumor #1 and 8.7% and 7.8% respectively for tumor #2 (Fig 7A, C, D). When compared to the no-antibody control, 13200 decreased tumor invasion by 77.8% (tumor #1) and 70.1% (tumor #2). Similarly, 12106 decreased invasion by 53.6% and 60.8% in tumors #1 and #2 respectively. Interestingly, both 13200 and 12106 were the autoantibodies that stained tumor organoids better than normal organoids. Purified IgG SLE1269 increased baseline invasion of tumor #1 to 55%, but decreased the baseline invasion of tumor #2 to 5.9% (Fig 7A, C, D).

We asked if the ability of autoantibodies to block invasion was dose dependent. We cultured organoids isolated from tumor #2 with a lower dosage (4% culture volume) of 13200 and 12106. The functional effects of the autoantibodies 13200 and 12106 are definitely dose dependent, with the lower dosages decreasing invasion by 56.5% and 44.4% respectively (Fig 7E). However, even this lower dose was sufficient to reduce tumor invasion compared to control autoantibodies.

In summary, autoantibodies alter tumor invasion in 3D organotypic cultures - some autoantibodies increase tumor invasion, while others decrease it.

3.4 Autoantibodies from No Cancer Patients Reduce Tumor Invasion More Effectively Than Their Cancer Counterparts

We next asked if the effect of serum autoantibodies on tumor invasion was dependent on the cancer status of the patient. Since the cancer statuses of patients from whom the previous set of autoantibodies were collected was unknown, we used purified IgGs 10015 (anti-TIF1g, no cancer), 9070 (anti-TIF1g, cancer), 7107 (anti-NXP2, no cancer), and 9109 (anti-NXP2, cancer) for this purpose.

The tumor (#3) used for this set of experiments was a ER+/PR+/HER2- breast tumor whose organoids were 20% invasive in the absence of any autoantibodies (Fig 8B). When cultured with 150ug/mL of control antibody C34, the invasion levels remained almost unchanged at 20.5% (Fig 8B, C). However, patient derived autoantibodies added at the same concentration altered tumor invasion in 3D culture. Antibodies derived from patients with cancer – 7107 (anti-NXP2) and 9070 (anti TIF1g) – increased tumor invasion to 20.9% and 25% respectively. On the other hand, IgGs isolated from autoimmune patients with no cancer – 9109 (anti-NXP2) and 10015 (anti TIF1g) – decreased tumor invasion to 12.1% and 18.4% respectively. All of the above autoantibodies had minimal cytotoxicity, ranging from 2-10% (Fig 8B, C).

In conclusion, autoantibodies isolated from patients with no cancer are more effective in reducing tumor invasion than those isolated from cancer patients.

CHAPTER 4: DISCUSSION AND FUTURE PERSPECTIVES

Despite the progress in our understanding of breast cancer development and progression, patients with metastatic breast cancer continue to have a five-year survival rate of 20%. Thus, there is an urgent need to better understand and treat metastasis. Cancer immunotherapy is gaining attention for its potential to turn our own immune system against cancer. However, drugs against cancer proteins within cells have not been developed because antibodies are considered too large to pass through the membrane of most cell types. Our research group has previously identified specialized cancer cells that commonly lead collective invasion in breast cancers (Cheung et al., 2013). These leader cells express keratin-14 (K14) and disrupting K14 expression potently blocks cancer invasion and metastasis. These studies indicate that targeting K14 might have therapeutic benefit. However, K14 is an intracellular cytoskeletal protein, raising the question how K14 might be targeted.

Autoantibodies are antibodies directed at self-antigens, typically in the context of autoimmune disease. An increase in the levels of autoantibodies precedes cancer development. Specific autoantibodies in scleroderma patients has been shown to be associated with an increased risk for cancer development (Shah et al., 2010), and in a subset of these patients, these autoantibodies recognize a somatically mutated gene expressed in the breast tumor (Joseph et al., 2014). Interestingly, lupus auto-antibodies against dsDNA have been shown to directly penetrate and kill cells (Hansen et al., 2012). This allows for development of drugs that travel through the bloodstream and specifically

target cells with certain intracellular proteins. Furthermore, recent unpublished data indicates that autoantibodies against K14, a gene important for collective tumor invasion, have been identified in the serum of patients with the autoimmune disease dermatomyositis. Here we test the hypothesis that autoantibodies from patients with autoimmune diseases regulate collective tumor invasion in 3D organotypic cultures.

Human tumor organoids have emerged as a powerful model for cancer biology. Although human tumor organoids offer unique opportunities for experimental therapeutics and personalized medicine, it remains unclear how to optimally isolate and analyze tumor organoids from patient samples obtained during routine clinical workflow. In this thesis, we report our experience developing an efficient methodology that allows us to move from patient samples to human tumor organoids to ex-vivo functional readouts for invasion. Using this framework, we uncover an unappreciated diversity of invasive behavior in human breast tumor samples. It is still unclear how invasion in collagen and K14 expression correlates with the survival and relapse information from these patients.

We then tested eighteen antisera obtained from patients with myositis, lupus and scleroderma, and two control antisera from normal patients, for their ability to specifically bind tumor cells in organoids generated from human mammary tumors. We showed positive cytoplasmic and/or nuclear binding of ten antisera to tumor organoids. Nuclear staining was always observed only in a fraction of the cells of the organoid. This is consistent with the expected heterogeneity in the tumor cells. Although control antisera did not bind tumor organoids, there was significant binding of these antisera with normal

mammary organoids. The finding that the level of IgG anti-mammary antibodies is high for control sera suggests that these are natural autoantibodies, rather than disease-specific autoantibodies. It is possible that the circulation of these normal antibodies is reduced in patients with autoimmune diseases. A similar result was found for patients with a chronic pulmonary disorder (Daffa et al., 2015). The above binding pattern could also be explained by reports suggesting that IgM autoantibodies in the sera of normal individuals reduce the reactivity of IgG autoantibodies, and this inhibition is removed when IgG is purified from these sera (Melero et al., 1997).

Two of these antisera we tested – 13200 and 12106 – were from patients with dermatomyositis and show higher specificity for tumor cells relative to normal mammary. The remaining antisera stain normal breast organoids better than the tumor; this could be because cancer cells commonly adopt several strategies to evade the immune recognition, and hence could fail to bind these autoantibodies.

In certain cases, multiple purified IgGs contained the same known antibodies, however, their staining pattern was not similar. Since the antibodies are not purified, the observed staining pattern could be because of the other antibodies in the serum. These 'different' antibodies could possess functional potential in blocking tumor progression.

The presence of certain autoantibodies in breast cancer patients have been associated with a better prognosis (Blixt et al., 2011), implicating a direct role of these autoantibodies in preventing tumor progression. Importantly, we have now shown that several serum autoantibodies reduce tumor invasion in 3D organotypic culture. IgG purified from

normal individuals increases the base line invasion compared to a no antibody control. Purified IgGs 13200 and 12106 function in a dose dependent fashion to reduce tumor invasion. Another autoantibody – SLE1269 – robustly increases invasion in one tumor, but decreases invasion effectively in the other. Since it has been shown that autoantibodies can be produced against antigens mutated in the tumor (Joseph et al., 2014), autoantibodies in SLE1269 could have been specific to antigens on one of the two tumors. The targets of these three IgGs may provide therapeutic targets for breast cancer. The nature of the functionally relevant autoantibodies from these sera that block tumor invasion could be established by cloning the B-cell receptor from blood cells collected from these patients, and testing the effect each clone on collective invasion in 3D organotypic cultures. Once we have a specific antibody of interest, we could evaluate its *in vivo* effects on tumor progression by using a human xenograft mouse model.

About 83% of patients with dermatomyositis that also develop cancer secrete autoantibodies against NXP2 and TIF1gamma (Fiorentino et al., 2013). We wanted to test if these autoantibodies were any different from the remaining 17% of patients in whom malignancy did not present itself. Interestingly, we observed that antibodies from no-cancer individuals is more effective in reducing tumor invasion than those obtained from cancer patients. This further implicates the role of the immune system in keeping cancer at bay. As hypothesized by Joseph et al. (Joseph et al., 2014), we believe that in autoimmune patients with no cancer, the immune system is actively editing the cancer and preventing its emergence.

Although it is now known that serum autoantibodies from patients with autoimmune diseases holds potential in preventing tumor progression, future efforts should be made to elucidate the molecular mechanisms by which this could be achieved. There are several studies that prove that serum autoantibodies can physically enter the cells and induce cell death (Hansen et al., 2012; Noble et al., 2014). It is worth testing if autoantibodies used in this study interfere with collective invasion by cell penetration. This could be accomplished by staining fixed tumor organoids treated with autoantibodies with goat anti- human secondary antibodies (Golan et al., 1993).

Development of tumor-selective therapies is a critical goal in cancer research. It has been shown that lupus autoantibodies can induce apoptosis in BRCA-deficient tumor cells (Hansen et al., 2012) and can sensitize certain cancer cells to anti-cancer drugs (Noble et al., 2014). It would also be interesting to see the effect of these selected autoantibodies on tumor invasion in the presence of an external anti-cancer therapy. Also, ex-vivo cultures described above were carried out with almost no stromal cells. How immune cells alter the effect of these antibodies still remains to be understood.

Since death due to breast cancer is, for the majority, restricted in patients with metastatic disease, it is important to develop therapeutic strategies that target this progression. Mutations in tumor antigens result in the production of antigenically distinct regions of the protein and are able to induce IgG autoantibodies. Several of these antibodies are useful in the detection of breast cancer months before the onset of clinical symptoms (Madrid and Maroun, 2011). Furthermore, the lower incidence of metastases and delay in

development of metastases in patients with high levels of autoantibodies suggests that the antibodies may play a role in inhibiting the progression of malignancy (Blixt et al., 2011). The goal of this thesis was to establish the functional potential for autoantibodies derived from patients with autoimmune disease to block tumor invasion in 3D culture. Novel results presented here suggest that autoantibodies could play a crucial role in controlling tumor progression by targeting genes that are critical to collective invasion (Fig 8). These experiments also reveal the potential for organoid assays to be used as a mechanism to identify therapeutic antibodies that target cancer. The precise mechanism by which this is achieved remains to be elucidated.

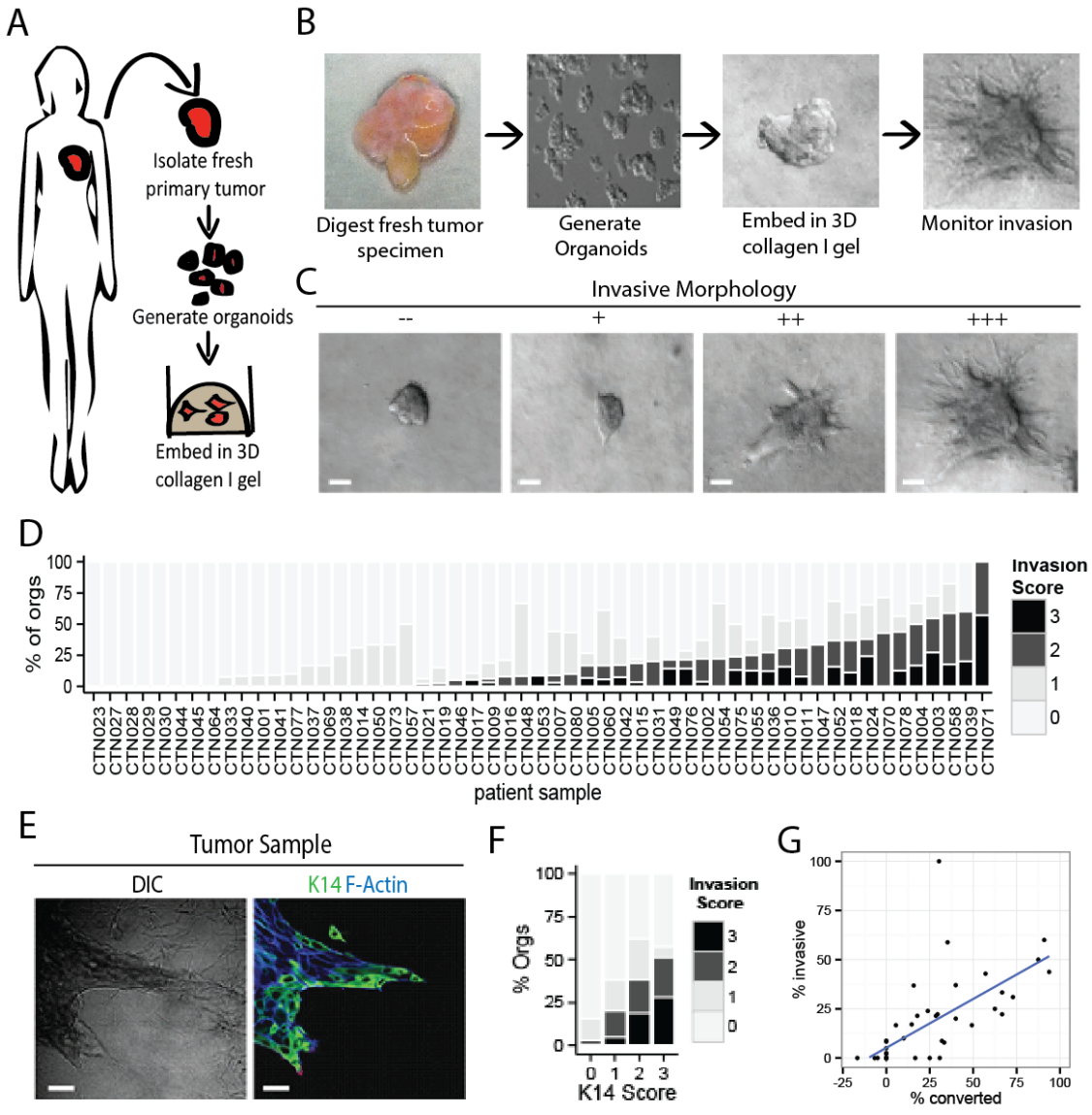
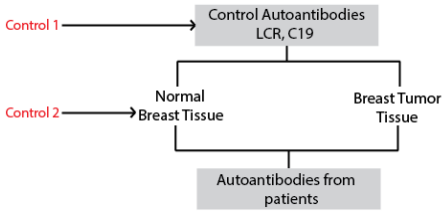


Fig 1: ***(A)*** *Workflow for isolation of tumor organoids from fresh patient samples.* ***(B)*** *Workflow of human tumor organoid generation with pictures taken during the experiment* ***(C)*** *Primary human tumor organoids were scored for invasive morphology into four categories.* ***(D)*** *Invasive morphology was scored on viable patient samples (N=30 patient samples; N=1139 organoids) and sorted from the least to most invasion.* ***(E)*** *Left panel: DIC image of a collectively invading strand of a tumor organoid into Collagen I. Right panel: Confocal image displaying the K14 expression in leader cells at the invasive front.* ***(F)*** *Strong positive correlation between the level of invasion and K14 expression in the organoids.* ***(G)*** *Strong positive correlation between the level of invasion and K14 conver sion.*

A



B

Disease	Total number of Antisera	Number of antisera that stained tumor organoids	Number of antisera that stained nomral organoids
Dermatomyositis	4	3	4
Scleroderma	9	4	8
Lupus	5	3	5

C

SNo.	Serum (IgG) #	Disease	Autoantibodies	TUMOR		NORMAL	
				Intensity of Cytoplasmic Staining	Intensity of Nuclear Staining	Intensity of Cytoplasmic Staining	Intensity of Nuclear Staining
1	LCR	control	none	-	-	+	-
2	C19	control	none	-	-	++	-
3	SLE 2055	lupus	Ro52 + La	++	++	++	++
4	FW-1366	scleroderma	RNA polymerase III	++	++	++	++
5	13,040	myositis	Ro52 + Jo1	++	-	++	-
6	13,200	myositis	NXP2	++	-	-	-
7	SLE 1269	lupus	DNA + Sm + RNP + Ro52 + La	++	-	++	++
8	13,142	myositis	Mi2	+	+	++	++
9	FW-1965	scleroderma	RNA polymerase III	+	+	+	+
10	12,106	myositis	TIF1gamma	+	+	-	+
11	SLE 1172	lupus	DNA + Sm + RNP	+	-	+	+
12	FW-1402	scleroderma	centromere	+	-	+	-
13	13,019	myositis	MDA5	-	-	+	-
14	12,094	myositis	TIF1gamma	-	-	+	-
15	13,070	myositis	MDA5	-	-	+	-
16	RFW-0042	scleroderma	topoisomerase 1	-	-	++	+
17	FW-1990	scleroderma	topoisomerase 1	-	-	++	-
18	SLE 1246	lupus	DNA + Sm + RNP	-	-	++	-
19	13,195	myositis	Mi2	-	-	+	-
20	13,093	myositis	NXP2	-	-	+	-

Fig 2: ***(A)*** *Schematic for immunofluorescence staining. Control 1: Staining with control antisera from normal patients, Control 2: Staining normal mammary organoids with all antisera.* ***(B)*** *Distribution of the total number of autoantibodies among the various disease types, and fraction of positive staining with normal breast and tumor organoids.* ***(C)*** *List of all antisera, disease type, known antibodies for each, and positivity of staining in the cytoplasm and nucleus against tumor and normal organoids. Positivity of staining was scored as -, + or ++.*

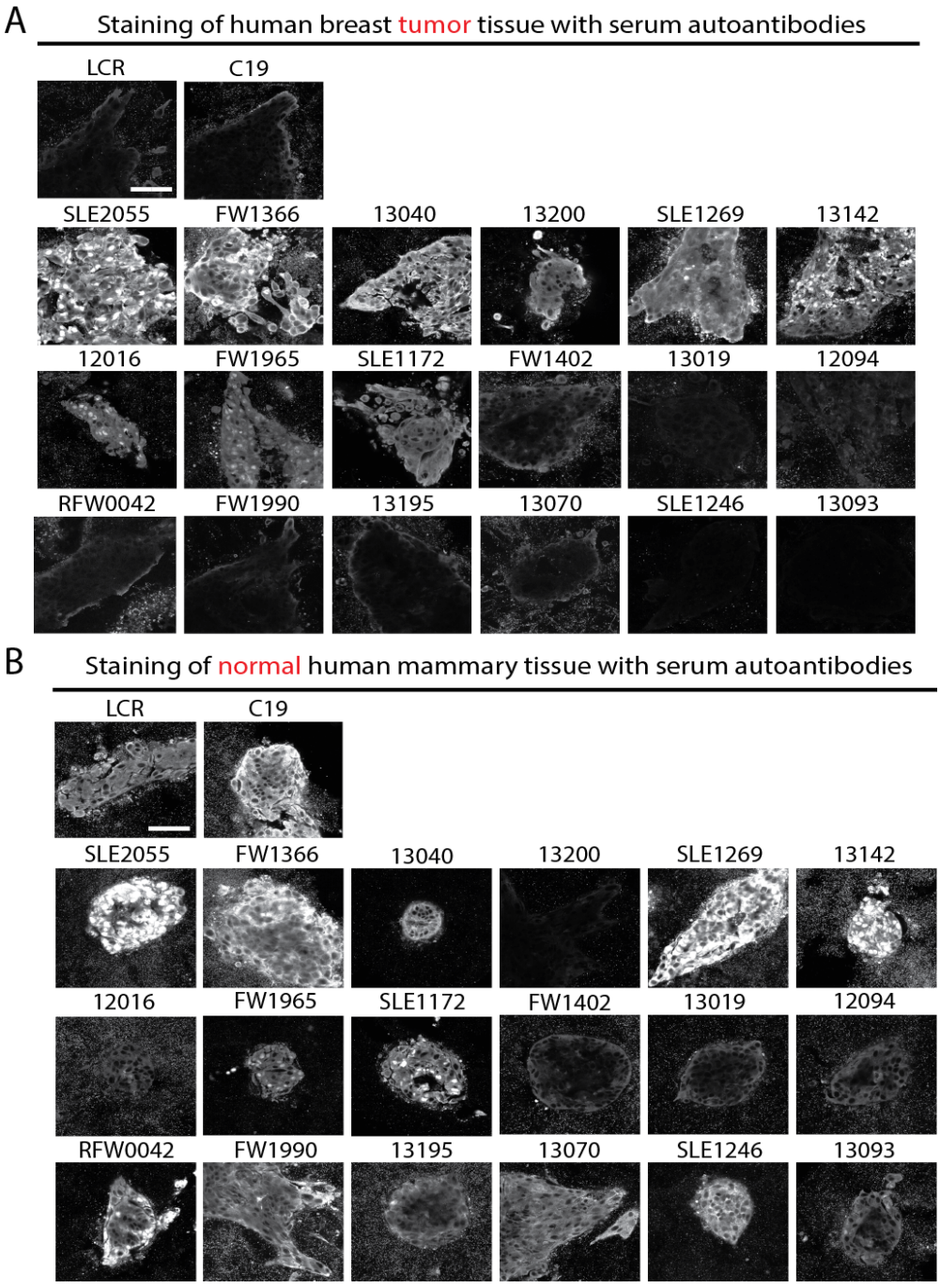


Fig 3: ***(A)*** *Representative images of staining results for two control antisera (C19, LCR) and eighteen patient antisera used to stain patient breast tumor organoids. Images are arranged in decreasing order of intensity.* ***(B)*** *Representative images of staining results for two control antisera (C19, LCR) and eighteen patient antisera used to stain normal breast organoids. Images are order matched to the panel above.*

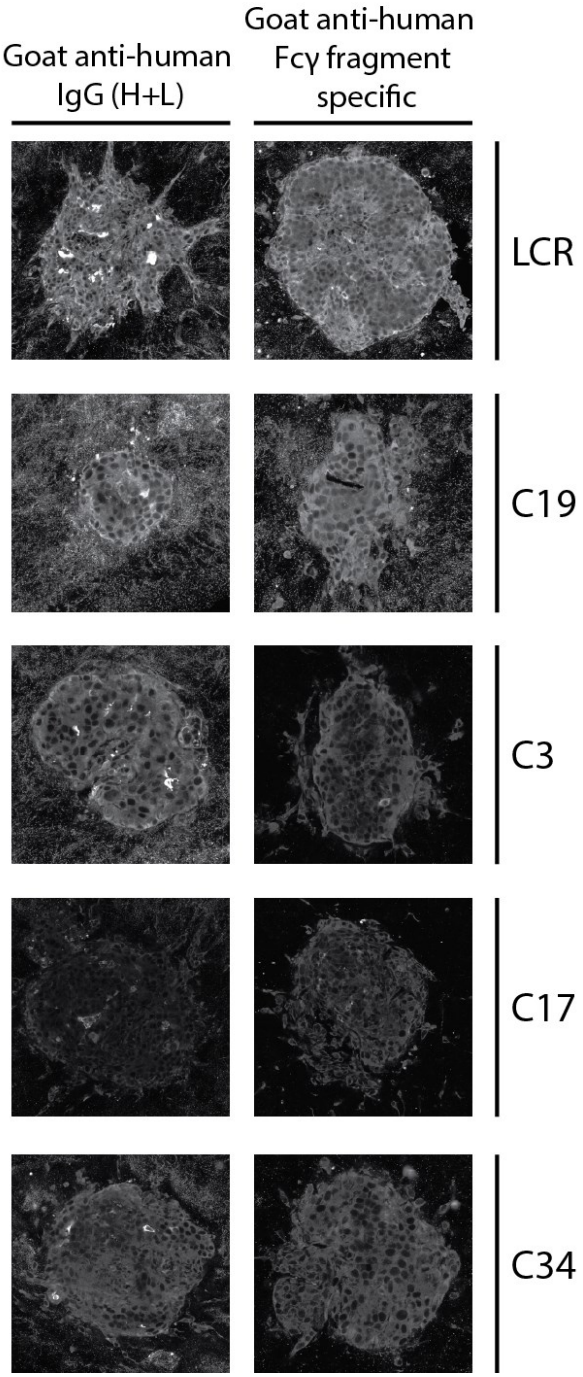


Fig 4: *Representative images of normal mammary organoids stained with control autoantibodies (LCR, C19, C3, C17 and C34). Staining procedures were completed with one of two secondary antibodies – goat anti-human IgG (H+L) or goat anti-human Fc-gamma specific IgG.*

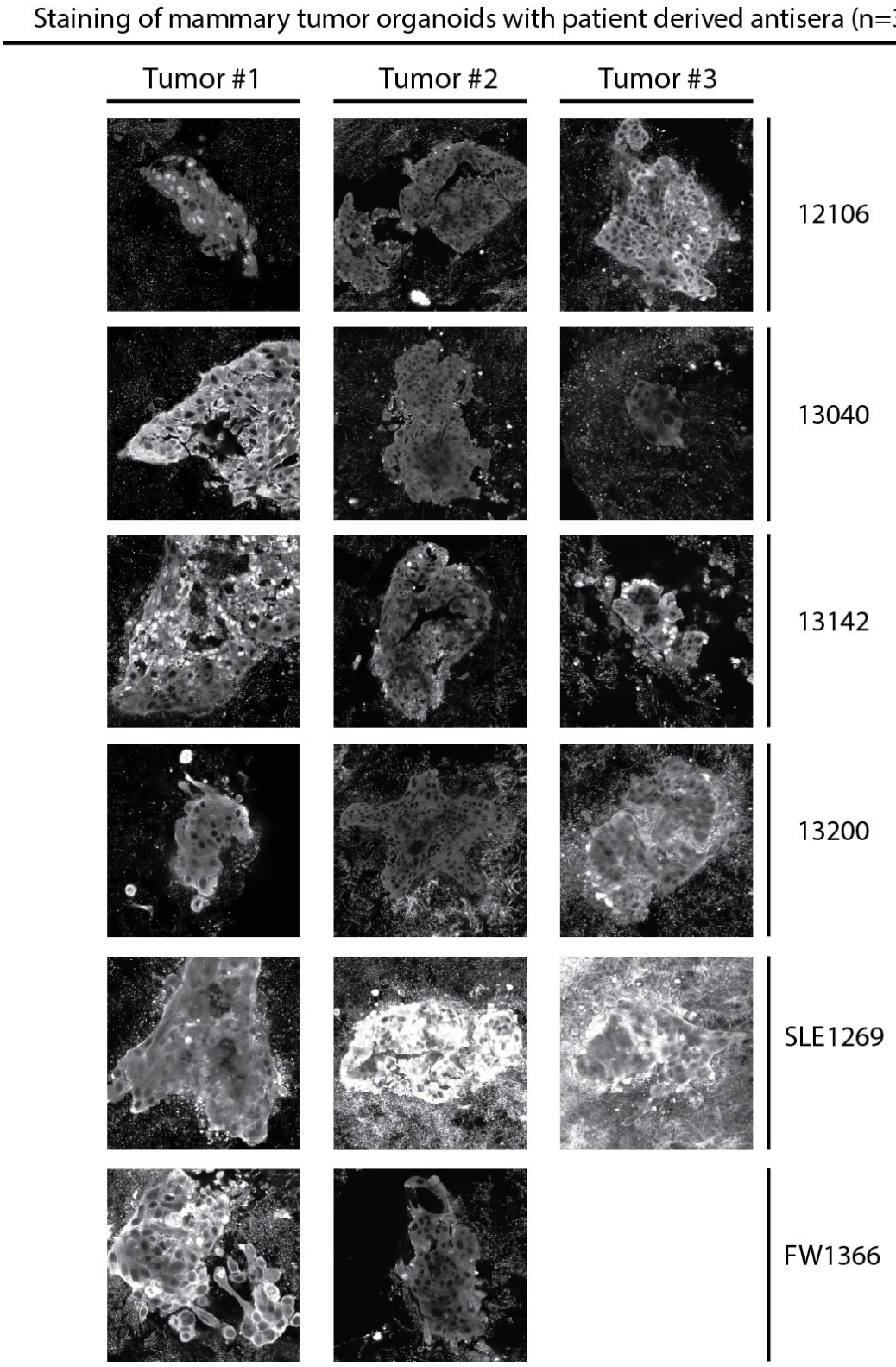


Fig 5: *Representative images of tumor organoids generated from three human breast tumors stained with patient derived autoantibodies (12106, 13040, 13142, 13200, SE1269, and FW1366).*

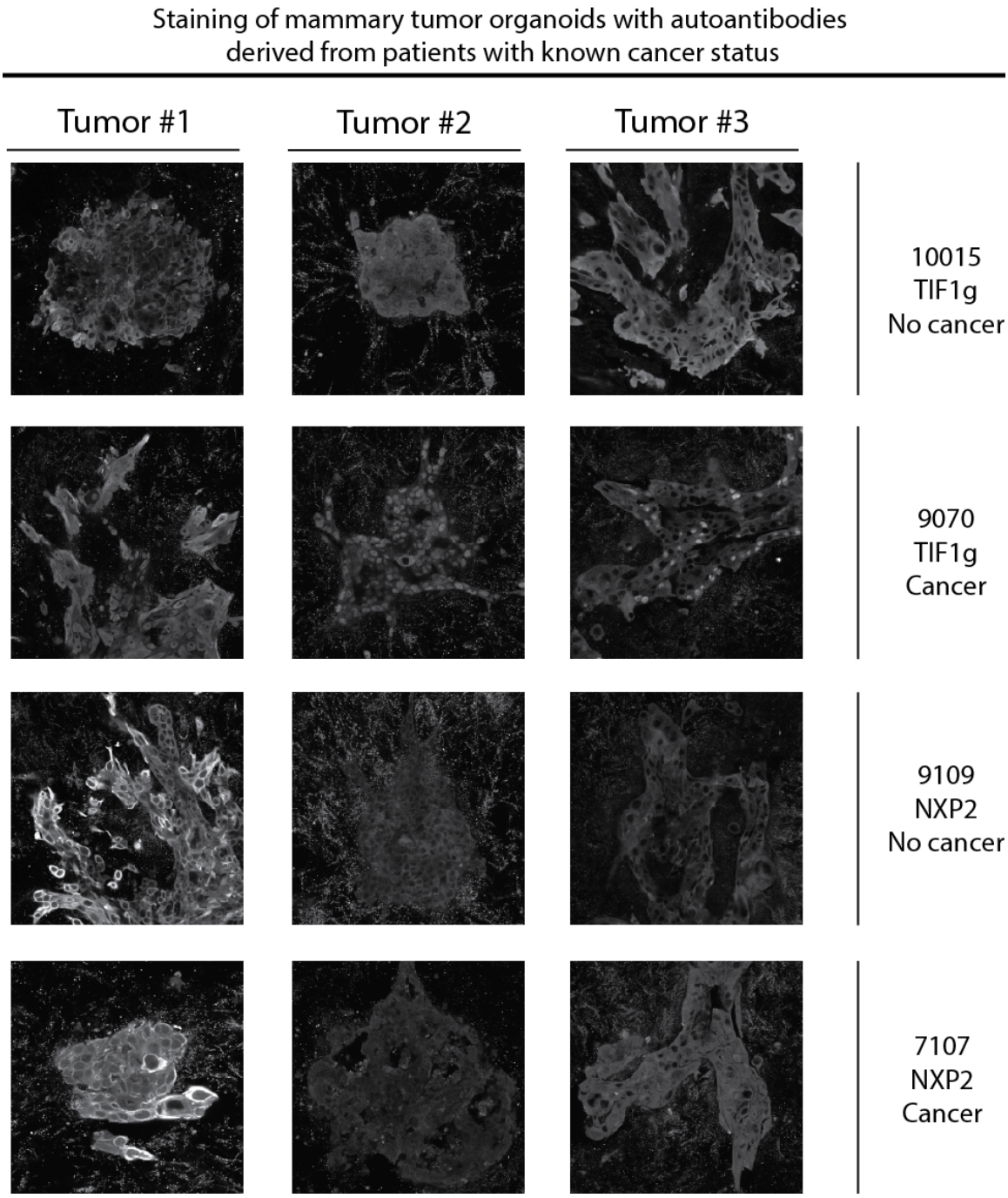
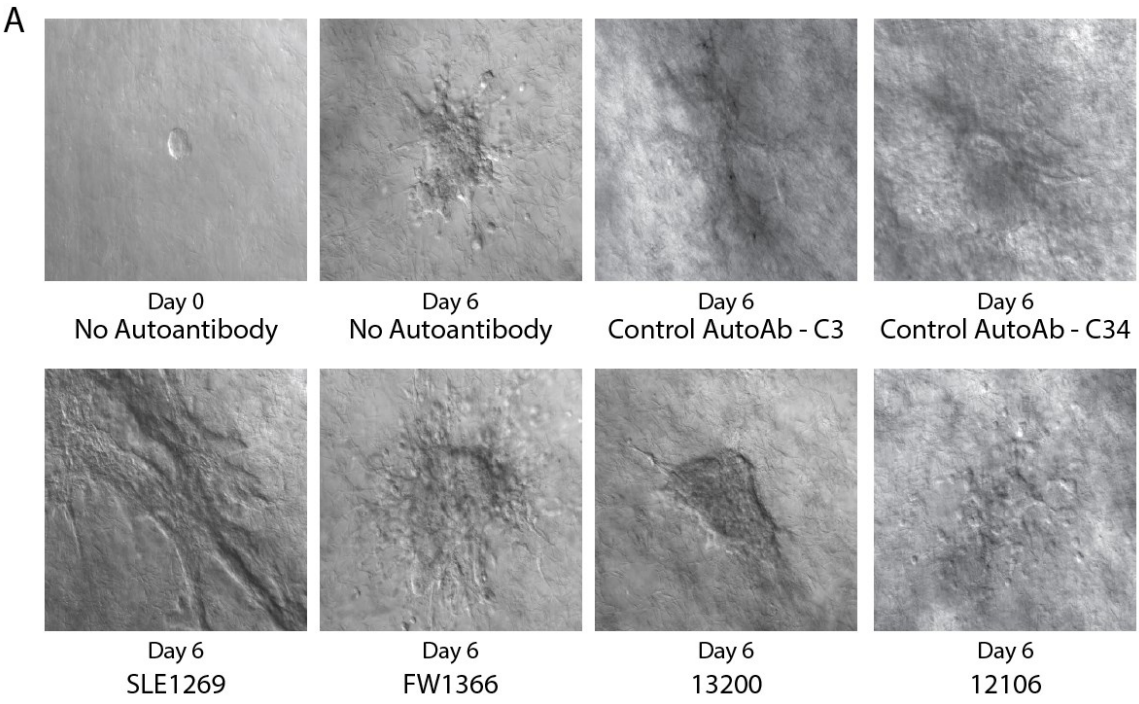
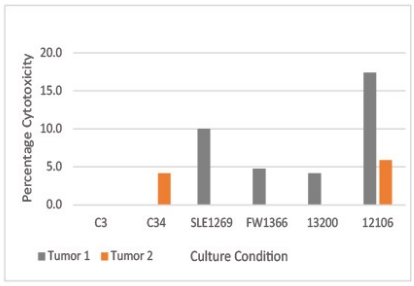


Fig 6: *Representative images of tumor organoids generated from three human breast tumors stained with patient derived autoantibodies. 10095 and 9070 are autoantibodies directed against TIF1 gamma purified from the serum of patients without or with cancer respectively. Similarly, anti-NXP2 autoantibodies 9109 and 7107 were purified from the serum of patients without or with cancer respectively.*



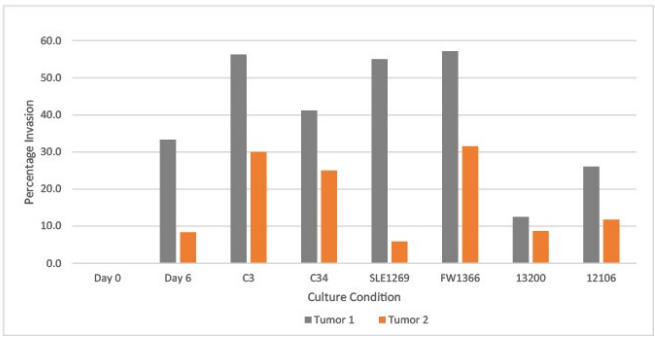
B



C

Culture Condition	% cytotoxicity		% invasive orgs		% decrease in invasion	
	Tumor #1	Tumor #2	Tumor #1	Tumor #2	Tumor #1	Tumor #2
Day 0	0.0	0.0	0.0	0.0	-	-
Day 6	0.0	0.0	33.3	8.3	-	-
C3	0.0	0.0	56.3	30.0	-	-
C34	0.0	4.2	41.2	25.0	-	-
SLE1269	10.0	0.0	55.0	5.9	2.3	80.4
FW1366	4.8	0.0	57.1	31.6	-1.5	-5.3
13200	4.2	0.0	12.5	8.7	77.8	71.0
12106	17.4	5.9	26.1	11.8	53.7	60.8

D



E

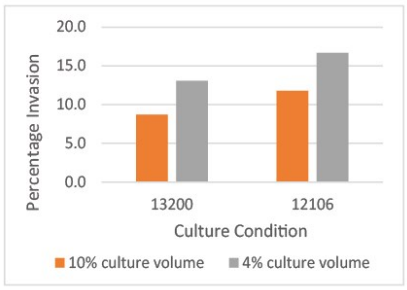
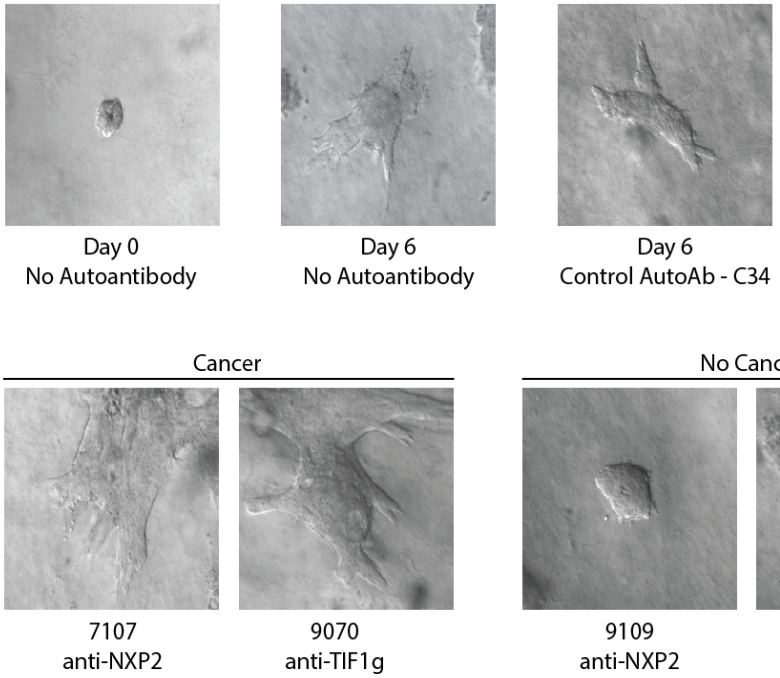


Fig 7: ***(A)*** *Effect of autoantibodies on tumor invasion in 3D organotypic culture. Representative DIC images depicting the invasion profile of tumor organoids collected on d0, or d6 without any autoantibody, or d6 with one of the following autoantibodies – SLE1269, FW1366, 13200, 12106, C33 or C34 (control autoantibodies).* ***(B)*** *Bar graph comparing the cytotoxicity of each*

autoantibody. ***(C)*** *Table summarizing the cytotoxicity, percentage invasion and percentage decrease in invasion for each autoantibody (n=2 tumors).* ***(D)*** *Bar graph comparing the effect of each autoantibody on collective invasion of tumor organoids derived from 2 tumors. (E) Dose-dependency of autoantibodies 13200 and 12106 in reducing tumor invasion.*

Effect of autoantibodies (obtained from patients with a known cancer status) on tumor invasion in 3D culture

A

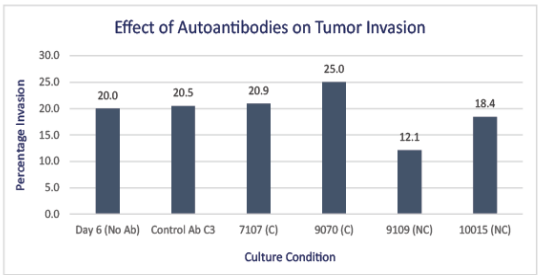


B

Culture Condition	Total no. of organoids	Number of organoids						% non-invasive	% invasive	% dead orgs
		Score = 3	Score = 2	Score =1	Score = 0	Score = b	Score = d			
Day 6 (No Ab)	50	6	4	6	29	1	4	70.0	20.0	8.0
Control Ab C3	39	5	3	3	26	1	1	74.4	20.5	2.6
7107 (C)	43	5	4	4	28	0	2	74.4	20.9	4.7
9070 (C)	40	7	3	2	24	0	4	65.0	25.0	10.0
9109 (NC)	33	1	3	3	24	0	2	81.8	12.1	6.1
10015 (NC)	38	5	2	3	25	0	3	73.7	18.4	7.9

Key: Organoids were assigned an invasion score of 0-3 based on the number of protrusive strands they extend into collagen; d: dead organoids; b: not analyzable. Organoids with a score of 0-1 and 2- 3 were grouped as non-invasive and invasive respectively. **NC, C: Ab isolated from patient with no cancer and cancer respectively.**

C



D

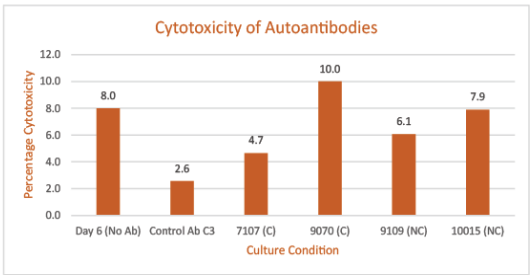


Fig 8: *Effect of patient cancer-status on autoantibody-mediated decrease in tumor invasion.* ***(A)*** *Representative DIC images depicting the invasion profile of tumor organoids collected on d0, or d6 without any autoantibody, or d6 with one of the following autoantibodies – 7107, 9070, 9109,*

10015 or C34 (control autoantibody). ***(B)*** *Table summarizing the invasion score, percentage invasion and cytotoxicity for each autoantibody (n=1 tumor).* ***(C)*** *Bar graph comparing the effect of each autoantibody on invasion.* ***(D)*** *Bar graph comparing the cytotoxicity of each autoantibody.*

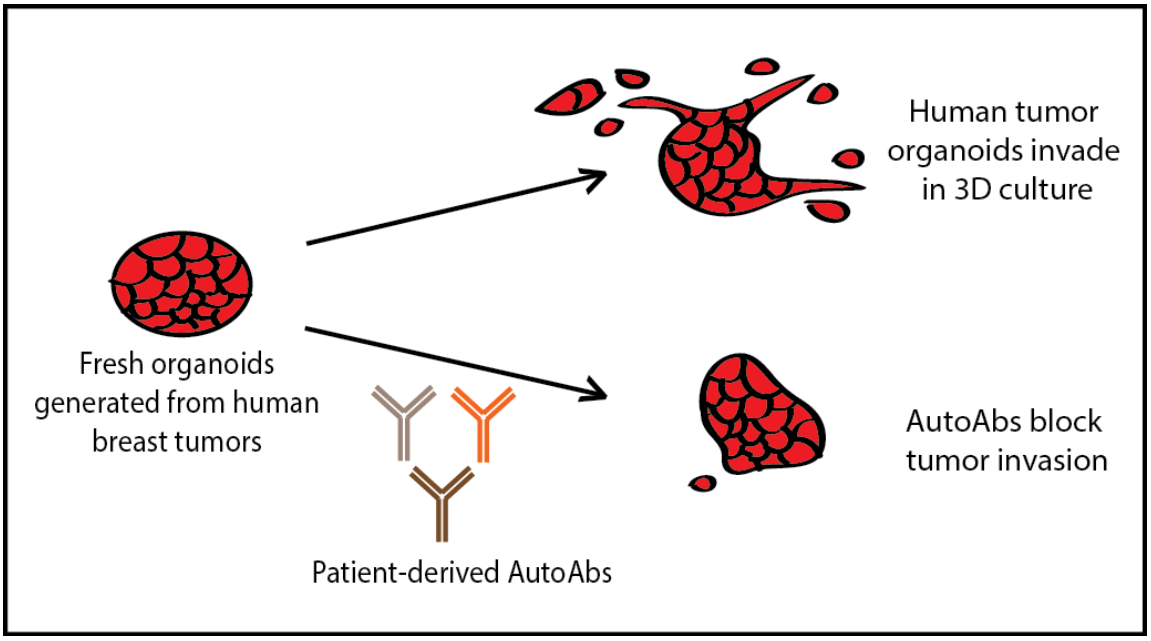


Fig 9: *Working model - Autoantibodies derived from patients with autoimmune diseases (AutoAbs) block tumor invasion in 3D organotypic cultures.*

REFERENCES:

1. (1998). Mastectomy or lumpectomy? The choice of operation for clinical stages I and II breast cancer. The Steering Committee on Clinical Practice Guidelines for the Care and Treatment of Breast Cancer. Canadian Association of Radiation Oncologists. CMAJ 158 Suppl 3, S15-21.
2. Alarcon-Segovia, D. (2001). The recovery of self tolerance in SLE. Lupus 10, 521-522.
3. Alarcon-Segovia, D., Ruiz-Arguelles, A., and Fishbein, E. (1978). Antibody to nuclear ribonucleoprotein penetrates live human mononuclear cells through Fc receptors. Nature 271, 67-69.
4. Allison, K.H. (2012). Molecular pathology of breast cancer: what a pathologist needs to know. Am J Clin Pathol 138, 770-780.
5. Avrameas, S. (1991). Natural autoantibodies: from 'horror autotoxicus' to 'gnothi seauton'. Immunol Today 12, 154-159.
6. Bernatsky, S., Ramsey-Goldman, R., Foulkes, W.D., Gordon, C., and Clarke, A.E. (2011). Breast, ovarian, and endometrial malignancies in systemic lupus erythematosus: a meta-analysis. Br J Cancer 104, 1478-1481.
7. Berry, D.A., Cronin, K.A., Plevritis, S.K., Fryback, D.G., Clarke, L., Zelen, M., Mandelblatt, J.S., Yakovlev, A.Y., Habbema, J.D., and Feuer, E.J. (2005). Effect of screening and adjuvant therapy on mortality from breast cancer. N Engl J Med 353, 1784-1792.
8. Blixt, O., Bueti, D., Burford, B., Allen, D., Julien, S., Hollingsworth, M., Gammerman, A., Fentiman, I., Taylor-Papadimitriou, J., and Burchell, J.M. (2011). Autoantibodies to

aberrantly glycosylated MUC1 in early stage breast cancer are associated with a better prognosis. Breast Cancer Res 13, R25.

9. Burnet, M. (1957). Cancer: a biological approach. III. Viruses associated with neoplastic conditions. IV. Practical applications. Br Med J 1, 841-847.
10. Chaffer, C.L., and Weinberg, R.A. (2011). A perspective on cancer cell metastasis. Science 331, 1559-1564.
11. Cheung, K.J., Gabrielson, E., Werb, Z., and Ewald, A.J. (2013). Collective invasion in breast cancer requires a conserved basal epithelial program. Cell 155, 1639-1651.
12. Chinoy, H., Salway, F., John, S., Fertig, N., Tait, B.D., Oddis, C.V., Ollier, W.E., and Cooper, R.G. (2007). Interferon-gamma and interleukin-4 gene polymorphisms in Caucasian idiopathic inflammatory myopathy patients in UK. Ann Rheum Dis 66, 970-973.
13. Cohen, I.R. (2000). Discrimination and dialogue in the immune system. Semin Immunol 12, 215-219; discussion 257-344.
14. Cohen, I.R., and Cooke, A. (1986). Natural autoantibodies might prevent autoimmune disease. Immunol Today 7, 363-364.
15. Cooper, G.S., Germolec, D., Heindel, J., and Selgrade, M. (1999). Linking environmental agents and autoimmune diseases. Environ Health Perspect 107 Suppl 5, 659-660.
16. Coutinho, A., Kazatchkine, M.D., and Avrameas, S. (1995). Natural autoantibodies. Curr Opin Immunol 7, 812-818.

17. Daffa, N.I., Tighe, P.J., Corne, J.M., Fairclough, L.C., and Todd, I. (2015). Natural and disease-specific autoantibodies in chronic obstructive pulmonary disease. Clin Exp Immunol 180, 155-163.
18. Danke, N.A., Koelle, D.M., Yee, C., Beheray, S., and Kwok, W.W. (2004). Autoreactive T cells in healthy individuals. J Immunol 172, 5967-5972.
19. Diefenbach, A., and Raulet, D.H. (2002). The innate immune response to tumors and its role in the induction of T-cell immunity. Immunol Rev 188, 9-21.
20. Dunn, G.P., Bruce, A.T., Ikeda, H., Old, L.J., and Schreiber, R.D. (2002). Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 3, 991-998.
21. Ehrenstein, M.R., and Notley, C.A. (2010). The importance of natural IgM: scavenger, protector and regulator. Nat Rev Immunol 10, 778-786.
22. Encinas, J.A., and Kuchroo, V.K. (2000). Mapping and identification of autoimmunity genes. Curr Opin Immunol 12, 691-697.
23. Ewald, A.J., Brenot, A., Duong, M., Chan, B.S., and Werb, Z. (2008). Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis. Dev Cell 14, 570-581.
24. Filion, M.C., Proulx, C., Bradley, A.J., Devine, D.V., Sekaly, R.P., Decary, F., and Chartrand, P. (1996). Presence in peripheral blood of healthy individuals of autoreactive T cells to a membrane antigen present on bone marrow-derived cells. Blood 88, 2144-2150.
25. Fiorentino, D.F., Chung, L.S., Christopher-Stine, L., Zaba, L., Li, S., Mammen, A.L., Rosen, A., and Casciola-Rosen, L. (2013). Most patients with cancer-associated

dermatomyositis have antibodies to nuclear matrix protein NXP-2 or transcription intermediary factor 1gamma. Arthritis Rheum 65, 2954-2962.

26. Fleming, J.N., and Schwartz, S.M. (2008). The pathology of scleroderma vascular disease. Rheum Dis Clin North Am 34, 41-55; vi.
27. Friedl, P., Locker, J., Sahai, E., and Segall, J.E. (2012). Classifying collective cancer cell invasion. Nat Cell Biol 14, 777-783.
28. Furst, D.E., Amato, A.A., Iorga, S.R., Bancroft, T., and Fernandes, A.W. (2012). Medical costs and health-care resource use in patients with inflammatory myopathies in an insured population. Muscle Nerve 46, 496-505.
29. Golan, T.D., Gharavi, A.E., and Elkon, K.B. (1993). Penetration of autoantibodies into living epithelial cells. J Invest Dermatol 100, 316-322.
30. Grabar, P. (1975). Hypothesis. Auto-antibodies and immunological theories: an analytical review. Clin Immunol Immunopathol 4, 453-466.
31. Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. Cell 144, 646-674.
32. Hansen, J.E., Chan, G., Liu, Y., Hegan, D.C., Dalal, S., Dray, E., Kwon, Y., Xu, Y., Xu, X., Peterson-Roth, E., et al. (2012). Targeting cancer with a lupus autoantibody. Sci Transl Med 4, 157ra142.
33. Hill, C.L., Zhang, Y., Sigurgeirsson, B., Pukkala, E., Mellemkjaer, L., Airio, A., Evans, S.R., and Felson, D.T. (2001). Frequency of specific cancer types in dermatomyositis and polymyositis: a population-based study. Lancet 357, 96-100.

34. Invernizzi, P. (2007). The X chromosome in female-predominant autoimmune diseases. Ann N Y Acad Sci 1110, 57-64.

35. Ippolito, A., Wallace, D.J., Gladman, D., Fortin, P.R., Urowitz, M., Werth, V., Costner, M., Gordon, C., Alarcon, G.S., Ramsey-Goldman, R., et al. (2011). Autoantibodies in systemic lupus erythematosus: comparison of historical and current assessment of seropositivity. Lupus 20, 250-255.

36. Jemal, A., Siegel, R., Xu, J., and Ward, E. (2010). Cancer statistics, 2010. CA Cancer J Clin 60, 277-300.

37. Joseph, C.G., Darrah, E., Shah, A.A., Skora, A.D., Casciola-Rosen, L.A., Wigley, F.M., Boin, F., Fava, A., Thoburn, C., Kinde, I., et al. (2014). Association of the autoimmune disease scleroderma with an immunologic response to cancer. Science 343, 152-157.

38. Kyewski, B., and Klein, L. (2006). A central role for central tolerance. Annu Rev Immunol 24, 571-606.

39. Lacroix-Desmazes, S., Kaveri, S.V., Mouthon, L., Ayouba, A., Malanchere, E., Coutinho, A., and Kazatchkine, M.D. (1998). Self-reactive antibodies (natural autoantibodies) in healthy individuals. J Immunol Methods 216, 117-137.

40. Leighton, J., Kalla, R.L., Turner, J.M., Jr., and Fennell, R.H., Jr. (1960). Pathogenesis of tumor invasion. II. Aggregate replication. Cancer Res 20, 575-586.

41. Lu, J., Steeg, P.S., Price, J.E., Krishnamurthy, S., Mani, S.A., Reuben, J., Cristofanilli, M., Dontu, G., Bidaut, L., Valero, V., et al. (2009). Breast cancer metastasis: challenges and opportunities. Cancer Res 69, 4951-4953.

42. Madrid, F.F., and Maroun, M.C. (2011). Serologic laboratory findings in malignancy. Rheum Dis Clin North Am 37, 507-525.

43. Mammen, A.L. (2010). Dermatomyositis and polymyositis: Clinical presentation, autoantibodies, and pathogenesis. Ann N Y Acad Sci 1184, 134-153.

44. Mathe, G. (1987). Passive, adoptive, and active immunotherapy: a review of clinical trials in cancer. Cancer Detect Prev Suppl 1, 279-290.

45. Mayes, M.D., Lacey, J.V., Jr., Beebe-Dimmer, J., Gillespie, B.W., Cooper, B., Laing, T.J., and Schottenfeld, D. (2003). Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population. Arthritis Rheum 48, 2246-2255.

46. Melero, J., Tarrago, D., Nunez-Roldan, A., and Sanchez, B. (1997). Human polyreactive IgM monoclonal antibodies with blocking activity against self-reactive IgG. Scand J Immunol 45, 393-400.

47. Miller, W.R. (2004). Biological rationale for endocrine therapy in breast cancer. Best Pract Res Clin Endocrinol Metab 18, 1-32.

48. Muramatsu, M., Sankaranand, V.S., Anant, S., Sugai, M., Kinoshita, K., Davidson, N.O., and Honjo, T. (1999). Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. J Biol Chem 274, 18470-18476.

49. Nguyen-Ngoc, K.V., Cheung, K.J., Brenot, A., Shamir, E.R., Gray, R.S., Hines, W.C., Yaswen, P., Werb, Z., and Ewald, A.J. (2012). ECM microenvironment regulates

collective migration and local dissemination in normal and malignant mammary epithelium. Proc Natl Acad Sci U S A 109, E2595-2604.

50. Noble, P.W., Young, M.R., Bernatsky, S., Weisbart, R.H., and Hansen, J.E. (2014). A nucleolytic lupus autoantibody is toxic to BRCA2-deficient cancer cells. Sci Rep 4, 5958.
51. Noseworthy, J.H., Lucchinetti, C., Rodriguez, M., and Weinshenker, B.G. (2000). Multiple sclerosis. N Engl J Med 343, 938-952.
52. Ochsenbein, A.F., Fehr, T., Lutz, C., Suter, M., Brombacher, F., Hengartner, H., and Zinkernagel, R.M. (1999). Control of early viral and bacterial distribution and disease by natural antibodies. Science 286, 2156-2159.
53. Pardoll, D.M. (2012). Immunology beats cancer: a blueprint for successful translation. Nat Immunol 13, 1129-1132.
54. Parish, C.R. (2003). Cancer immunotherapy: the past, the present and the future. Immunol Cell Biol 81, 106-113.
55. Sahai, E. (2007). Illuminating the metastatic process. Nat Rev Cancer 7, 737-749.
56. Schreiber, R.D., Old, L.J., and Smyth, M.J. (2011). Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 331, 1565-1570.
57. Seddon, B., and Mason, D. (2000). The third function of the thymus. Immunol Today 21, 95-99.
58. Seidel, U.J., Schlegel, P., and Lang, P. (2013). Natural killer cell mediated antibody-dependent cellular cytotoxicity in tumor immunotherapy with therapeutic antibodies. Front Immunol 4, 76.

59. Shah, A.A., Hummers, L.K., Casciola-Rosen, L., Visvanathan, K., Rosen, A., and Wigley, F.M. (2015). Examination of autoantibody status and clinical features associated with cancer risk and cancer-associated scleroderma. Arthritis Rheumatol 67, 1053-1061.

60. Shah, A.A., Rosen, A., Hummers, L., Wigley, F., and Casciola-Rosen, L. (2010). Close temporal relationship between onset of cancer and scleroderma in patients with RNA polymerase I/III antibodies. Arthritis Rheum 62, 2787-2795.

61. Shamir, E.R., and Ewald, A.J. (2014). Three-dimensional organotypic culture: experimental models of mammalian biology and disease. Nat Rev Mol Cell Biol 15, 647-664.

62. Simian, M., Hirai, Y., Navre, M., Werb, Z., Lochter, A., and Bissell, M.J. (2001). The interplay of matrix metalloproteinases, morphogens and growth factors is necessary for branching of mammary epithelial cells. Development 128, 3117-3131.

63. Sinha, A.A., Lopez, M.T., and McDevitt, H.O. (1990). Autoimmune diseases: the failure of self tolerance. Science 248, 1380-1388.

64. Somers, E.C., Marder, W., Cagnoli, P., Lewis, E.E., DeGuire, P., Gordon, C., Helmick, C.G., Wang, L., Wing, J.J., Dhar, J.P., et al. (2014). Population-based incidence and prevalence of systemic lupus erythematosus: the Michigan Lupus Epidemiology and Surveillance program. Arthritis Rheumatol 66, 369-378.

65. Soussi, T. (2000). p53 Antibodies in the sera of patients with various types of cancer: a review. Cancer Res 60, 1777-1788.

66. Street, S.E., Cretney, E., and Smyth, M.J. (2001). Perforin and interferon-gamma activities independently control tumor initiation, growth, and metastasis. Blood 97, 192-197.

67. Stutman, O. (1975). Immunodepression and malignancy. Adv Cancer Res 22, 261-422.

68. Tevaarwerk, A.J., Gray, R.J., Schneider, B.P., Smith, M.L., Wagner, L.I., Fetting, J.H., Davidson, N., Goldstein, L.J., Miller, K.D., and Sparano, J.A. (2013). Survival in patients with metastatic recurrent breast cancer after adjuvant chemotherapy: little evidence of improvement over the past 30 years. Cancer 119, 1140-1148.

69. Tinoco, G., Warsch, S., Gluck, S., Avancha, K., and Montero, A.J. (2013). Treating breast cancer in the 21st century: emerging biological therapies. J Cancer 4, 117-132.

70. Tomer, Y., and Shoenfeld, Y. (1988). The significance of natural autoantibodies. Immunol Invest 17, 389-424.

71. Vargo-Gogola, T., and Rosen, J.M. (2007). Modelling breast cancer: one size does not fit all. Nat Rev Cancer 7, 659-672.

72. Walker, L.S., and Abbas, A.K. (2002). The enemy within: keeping self-reactive T cells at bay in the periphery. Nat Rev Immunol 2, 11-19.

Veena Padmanaban

Phone: 916-934-4148 |**Email**: vpadman4@jhu.edu

Education:

Johns Hopkins University, Homewood Campus, Baltimore, MD **(Aug2013-May2015)**
MS Biotechnology, *GPA 4.0*
Thesis: Harnessing patient derived autoantibodies to disrupt breast cancer invasion and metastasis.

SRM University (India's No.1 private university), Chennai, India **(Aug2009- May2013)**
B.tech Biotechnology, *GPA 9.41*
Honors: *Best outgoing student 2013 (Biotechnology), Founder's Scholarship (awarded to <1% of students), Six performance-based scholarships.*
Presented posters at the International Conference in Bioengineering (2010) National Conference on Frontiers in Biotechnology (2012, 2013) held at SRM University.

University of California, Davis, CA **(Sept 2011- Dec 2011)**
Fifth semester – Student Abroad Program, ***GPA 3.72***
Honors: *Certificate for academic excellence*

Research Experience:

Research Assistant, Johns Hopkins School of Medicine, Baltimore, MD **(Jan2014-Present)**

- *Guide: Dr. Andrew Ewald, Associate Professor, Department of Cell Biology*
  - Studying the role of collective epithelial dissemination in breast cancer metastasis. ***(manuscript under consideration at Nature).***
    Using a common metastatic breast cancer mouse model, it was shown that about 98% of the lung metastases arise from collective rather than single cell seeding events.
  - Understanding cancer invasion in human mammary tumor samples (*manuscript in preparation*).
    Human mammary tumors show a varying degrees of invasion in 3D collagen I gels. Invasion was found to be directly correlated with expression of keratin 14.
  - Studying the ability of serum autoantibodies from patients with autoimmune disease to block breast cancer invasion and metastasis.

Research Assistant, Johns Hopkins School of Medicine, Baltimore, MD **(Sept2013-Jan2014)**

- *Guide: Dr. Saraswati Sukumar, Co-Director, Breast Cancer Research Program, Sidney Kimmel Comprehensive Cancer Center*

  Worked on a project to block the interaction between mesenchymal stem cells and breast cancer stem cells, resulting in an increased sensitivity to epigenetic therapy against triple negative breast cancer.

Research Assistant, Indian Institute of Technology (IIT), Madras, India **(Dec2012- May2013)**

- *Guide: Dr. Nitish Mahapatra, Associate Professor, IIT, Madras*

  Worked on project to discover single nucleotide polymorphisms (SNPs) in the Chromogranin A gene promoter in an Indian population and evaluate their association with essential hypertension. *Identified eight SNPs in the promoter of Chromogranin A, four of which were associated with essential hypertension*

Research Assistant, University of California, Davis, CA **(Sept2011- Dec2011)**

- *Guide: Dr. Venkatesan Sundaresan, Professor, Department of Plant Biology*

  Studying the role of auxin and cytokinin signaling in the female gametophyte development in Arabidopsis.

Defense Research Laboratory, Ministry of Defense, Govt. of India **(December 2010)**

- *Guide: Dr. Sibnarayan Dutta, Scientist, Biotechnology Division*

  Completed a training on the rapid detection of Human Palpilloma virus (Genotype 16) on various clinical samples using Polymerase Chain Reaction (PCR).

Industrial Training:

Cancer Institute, Adayar, Chennai, India **(June 2012)**

- Completed training in the Department of Electron Microscopy – learnt techniques like processing tissue specimens for Electron Microscopy, creating of ultra-thin sections, and staining.
- Completed training in the Department of Cytogenetics – learnt tissue culture techniques, chromosomal preparations, G- Banding, Karyotyping and its analysis from human bone marrow samples using IKAROS software.

Laboratory techniques:

Mammalian cell culture, Mammary transplants on mice, DIC and Confocal Imaging, 3D organotypic culturing, Tail-vein injections in mice, Mice dissection, Cryosectioning, DNA and RNA isolation, Gene cloning, PCR, qPCR, Restriction digestion, Ligation, Western Blotting, Flow cytometry, Gel Electrophoresis, Immunohistochemistry assays, ELISA, Transfection.

Computer Skills:

Microsoft Office (Word, Excel, Outlook, Powerpoint), Image analysis software (ImageJ, Adobe Photoshop and Illustrator), Bioinformatics tools (Autodock, BLAST, FASTA, Clustal W2, Rasmol).

Publications:

Cheung, K. J., **Padmanaban, V.**, Silvestri, V., Schipper, K., Fairchild, A. N., Ewald, A. J. "Distant Metastasis Occurs Through Collective Epithelial Dissemination." *(Under Review at Nature, November 2014)*

Cheung, K. J., **Padmanaban, V**., Ewald, A. J. "The Spectrum of Invasion in Organoids from Primary Human Breast Tumors." *(Under preparation)*

Published Abstracts

Cheung, K. J., **Padmanaban, V.**, Silvestri, V., Schipper, K., Ewald, A. J. "Multiclonal seeding is a frequent route to metastatic spread." Poster, Philadelphia, PA. *American Society for Cell Biology (ASCB), 2014.*

Padmanaban, V., Cheung, K.J., Rosen, A., Rosen, L.V., Ewald, A.J. "Patient Derived Autoantibodies Bind to Antigens on Human Breast Tumor Organoids." Poster, Baltimore, MD. *Biotechnology symposium, Johns Hopkins University, 2014.*

Teaching and Leadership Experience:

- **New Student Representative** (Jan2014-Present)
  Indian Graduate Student Association, Johns Hopkins University
- **Coordinator, Milan 2013** (Dec2013-Apr2013)
  Conducted an ISO 9001-2000 certified student organized cultural festival of SRM University with a team of 13 students.
- **Coordinator, Bionicade 2013** (Nov2012-Jan2013)
  Conceptualized and organized the first ever edition of the Department of Biotechnology's national level technical festival.
- **Volunteer, Teaching, National Service Scheme** (Aug2009-Apr2010)
  Served as a mathematics instructor for 45 class 11 (high school junior) underprivileged students

Relevant Coursework:

- Cell Biology
- Molecular Biology
- Cancer Biology
- Immunology
- Biostatistics
- Biochemistry
- Bioassay Development
- Analytical Techniques in Biotechnology
- Genetic Engineering
- Biophysics
- Molecular Modelling and Drug Design
- Genomics and Proteomics